CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

21-368

PHARMACOLOGY REVIEW

GENERAL PHARMACOLOGY/TOXICOLOGY COVER SHEET

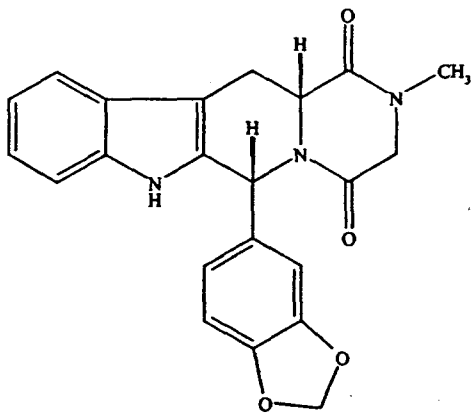
NDA No: 21-368 **Review No:** 1
Sequence No: 000 **Date/type of submission:** June 28, 2001/Original
Information to sponsor: Yes (x) No ()
Sponsor: Lilly ICOS LLC, Eli Lilly & Company, Indianapolis, IN 46285
Manufacturer for drug substance: Eli Lilly & Co., Tippecanoe Laboratories, Lafayette, IN 47909

Reviewer: Yangmee Shin, Ph.D.
Division: Division of Reproductive and Urologic Drug Products, HFD-580
Review completion date:

Drug:

Trade name: Cialis **Generic name:** Tadalafil
Code name: IC351 (LY450190)
Chemical name: Pyrazino[1',2':1,6]pyrido[3,4-b]indole-1,4-dione, 6-(1,3-benzodioxol-5-yl)-2,3,6,7,12,12a-hexahydro-2-methyl-, (6R-12aR)-

CAS registry No: 171596-29-5 **Mole file No:**
Molecular formula: $C_{22}H_{19}N_3O_4$ **Molecular weight:** 389.41
Structure:



Relevant INDs/NDAs/DMFs: IND 54,553 .
Drug class: β-carboline phosphodiesterase (PDE) type 5 inhibitor
Indication: Erectile Dysfunction (ED)

Clinical formulation: Yellow, film-coated, almond-shaped tablets containing 20 mg of tadalafil and inactive ingredients of lactose monohydrate, hydroxypropyl cellulose, hydroxypropyl methylcellulose, iron oxide, croscarmellose sodium, sodium lauryl sulfate, microcrystalline cellulose, talc, titanium dioxide, triacetin & magnesium stearate.

Route of administration: Oral

Proposed clinical use: Treatment of erectile dysfunction

Disclaimer: Tabular and graphical information is from sponsor's submission unless stated otherwise.

Studies reviewed within this submission:

PHARMACOLOGY (#S21422, #01-0007-11, #00-0010-11, #00-0009-11)
TOXICOKINETICS (#D01899, # — 88780, # — 88779, # —-353016)
TOXICOLOGY
1-Year oral toxicity study in beagle dogs (vol. 31, p 1, #D01899)
CARCINOGENICITY
2-Year oral carcinogenicity study in CD-1 mice (vol. 33, p 1, # — 88455)
2-Year oral carcinogenicity study in l— Wistar rats (vol. 34, p 1, # — 88203)
REPRODUCTIVE TOXICOLOGY
Segment II & III reproductive study in CD rats (vol. 37/38, p 1, # — -353010,—-353016)

Studies <u>not</u> reviewed within this submission (Appendix I):

<u>IND 54,553 Review #1, May 26, 1998</u>
PHARMACOLOGY (#97-001-14, #97002-14, #97-003-14, # — 94-007)
SAFETY PHARMACOLOGY (#20215, #S20996, #21011, #S20222, #97-004-14)
PHARMACOKINETICS
Absorption (#R21147, #D21148, #BPW662, #BPW641/BPW659)
Metabolism (#BPW549/BPW564, #BPW641/BPW659)
Distribution (#BPW618)
Protein binding (#BPW507, #BPW495)
TOXICOKINETICS (#R20861, #R21236, #D21148, #D20786, #D20863, #D21235)
TOXICOLOGY
Acute toxicity (#M20798, #M20799, #M20977, #M20978, #R20796, #R20797, #R20979, #R20980)
Repeated toxicity
1. Maximum repeatable daily oral dosage study in the — Wistar Rat (#R20791)
2. 1-Month oral toxicity study in — Wistar Rats (#R20861)
3. Study to determine the maximum repeatable daily oral dosage in the beagle dog (#D20786)
4. 1-Month oral toxicity study in the beagle dog (#D20863)
5. 6-Month oral toxicity study in the beagle dog (#D21235)

GENETIC TOXICOLOGY
1. Microbial mutagenicity study (#U20206)
2. Mouse lymphoma thymidine kinase mammalian cell mutation study (#V21166)
3. *In vitro* cytogenetic evaluation in cultured human lymphocytes (#V20918)
4. WHO nitrosation assay (#U21004)

<u>IND 54,553 Review #2</u>
TOXICOLOGY
1. 6-Month oral toxicity study in the beagle dog (#D21235)

<u>IND — Review #1, May 27, 1999</u>
PHARMACOKINETICS
Metabolism (#1999IV-RSL05, #006R00)
Excretion (#BPW549/BPW564)
TOXICOKINETICS (#88270)
TOXICOLOGY
1. 3-Month oral pilling toxicity study with a 13-week recovery period in the Beagle dog (#88270)

GENETIC TOXICOLOGY
1. Micronucleus assay in bone marrow of male — Wistar Rats (#R20937)

<u>IND — Review #2, Jul 26, 1999</u>
SAFETY PHARMACOLOGY (#PG9927)
TOXICOKINETICS (#M04298, #R18498, #M04398, # — -353004, # — -353005)
TOXICOLOGY
1. 1- & 3-Month oral gavage toxicity in CD-1 mice (#M04298)

REPRODUCTIVE TOXICOLOGY
1. Embryo/fetal development in CD-1 mice (# —353004)
2. Embryo/fetal development in CD rats (# —353005)

IND 54,553 Review #3, Aug 10, 1999
PHARMACOLOGY (#98-0001-11, #98-0002-11, #98-0003-11)
PHARMACOKINETICS (#132R98, #R14998, #M04198, ADME#6, ADME#7)
TOXICOKINETICS (# 88440, #21236)
TOXICOLOGY
1. 3-Month oral gavage toxicity in CD-1 mice (#88437)
2. 6-Month oral gavage toxicity in Wistar rats (#21236)

REPRODUCTIVE TOXICOLOGY
1. Oral gavage fertility study in CD rats (#96364)

JUSTIFICATION FOR 2-YEAR CARCINOGENICITY STUDY DOSE SELECTIONS IN RATS & MICE

IND — Review #3, Sep 3, 1999
PHARMACOLOGY (#PR9902)
PHARMACOKINETICS (#B00199, #R18498)
TOXICOKINETICS (#R18498, 88632)
TOXICOLOGY
1. 3-Month oral gavage in Fisher 344 rats (#R18498)
2. 6-Month oral pilling toxicity study with a 3-month recovery period in the Beagle dog (88632)

SPECIAL TOXICOLOGY
1. *In vitro* ocular irritation-agar diffusion cytolysis & aqueous pH in cultured rabbit cornea cells (#990416ADC)

IND — Review #4, Dec 27, 1999
PHARMACOLOGY (#PR9902)
SPECIAL TOXICOLOGY
1. *In vivo* eye irritation study in New Zealand White rabbits (#SLI3130.495)
2. *In vivo* acute dermal toxicity in New Zealand White rabbits (#SLI3130.487)

IND — Review #5, Aug 15, 2000
PHARMACOLOGY (#1999IV-EI004, #PR9906, #99-0005-11)
PHARMACOKINETICS
Distribution (#003R00)
Metabolism (#1999IV-SF038)
Excretion (#078R99, #002R00)
Repeat Dose in Monkeys (88548)

Introduction and drug history: IC351 is a potent, competitive and reversible inhibitor of cGMP specific PDE type 5 for an indication of ED under IND 54,553. It is also currently being investigated for — under IND — Major toxicities are irreversible seminiferous testicular atrophy and vasculitis in dogs. The original submission by ICOS Corporation was placed on clinical hold because of vasculitis findings in dogs and the high daily clinical dose up to 100 mg.

EXECUTIVE SUMMARY

I. Recommendations

A. Recommendation on Approvability: The preclinical studies conducted support the safety of the proposed dose of 20 mg of Cialis.

B. Recommendation for Nonclinical Studies: The 2-year carcinogenicity studies in male rats, and male and female mice were conducted at doses below those recommended by the ICH guidelines (see Exectutive CAC minutes in appendex II) based on the AUC exposures for the 20 mg human dose. The Committee recommended an additional alternative mouse carcinogenicity assay be conducted for Phase IV commitment unless the sponsor provided evidence for saturation of absorption by measuring either total radioactivity or metabolites.

C. Recommendations on Labeling: Refer to the labeling comments.

II. Summary of Nonclinical Findings

A. Brief Overview of Nonclinical Findings: Effective antihypertensive oral doses of IC351 were 1 mg/kg in the rat. Reduction in mean blood pressure occurred at doses from 20 mg/kg without effects on heart/respiration rate in conscious dogs, but moderate tachycardia was seen in a dog (1/2) at 30 mg/kg and in both dogs at 100 mg/kg in another study. The cardiovascular effects were not observed in the repeated toxicity studies. IC351 potentiated atrial natriuretic factor (ANF)-induced diuresis and natriuresis in rats at lower doses (0.1 mg/kg, i.v.) than those required for decreasing blood pressure. Slight-to-moderate ptosis and depression of the pinnal reflex were observed in rats given 200 mg/kg. IC351 did not cause death up to 2,000 mg/kg (p.o.) in mice and rats in acute studies. Like other PDE5 inhibitors, the major findings of IC351 treatment in repeated dose studies are arteritis and testicular degeneration/atrophy observed in multiple species. IC351 was not genotoxic and the carcinogenicity studies were negative although hepatocellular adenomas/carcinomas were observed with increased frequency in high dose male mice and rats. Reproductive and developmental studies in mice and rats displayed no adverse effects on fertility at doses up to 1,000 mg/kg. Mice were used for a second rodent species of embryo/fetal development studies since plasma exposure for rabbits was minimal. A NOAEL for maternal toxicity was established at 1,000 mg/kg in mice and 200 mg/kg in rats (based on reduced body weight gain). A NOAEL for F1 developmental toxicity in the rat could not be identified due to significantly reduced postnatal survival in all dose groups from the combined segment II/III study. Sponsor defined a NOAEL of 30 mg/kg from a subsequent study, which gives 9-fold exposure for the unbound parent drug (pregnant rat) to the human exposure at 20 mg. IC351 is a mild ocular and dermal irritant in New Zealand White rabbits.

B. Pharmacologic Activity: IC351 is a potent and selective inhibitor of PDE5 among the PDEs tested *in vitro*. PDE5 is a major cGMP-hydrolyzing enzyme in human cavernosal smooth muscle, and the inhibition of PDE5 by IC351 enhances relaxant effects of NO by stimulating cGMP levels. This leads to relaxation of penile resistance arteries and the smooth muscle to enhance the erectile response. IC351 strongly potentiated the inhibitory effects of sodium nitroprusside (SNP) on human platelet aggregation with complete inhibition at 0.25 μM, and on increased cGMP levels in human cavernosal smooth muscle, suggesting that the PDE5 inhibition by IC351 may lead to large increases in cGMP levels once activated. IC351 retains relatively low selectivity for PDE5 vs. human PDE11A (abstract from Am. Coll. Clin. Pharmacol., VA, 2001), which was widely expressed in kidney, liver, pituitary/salivary glands and testis (PNAS 97: 3702, 2000). Thus, pharmacological characterization of IC351 on human PDE11A may provide additional information on the mechanism of IC351.

C. Nonclinical Safety Issues Relevant to Clinical Use:

1. Testicular degeneration/atrophy were observed with increased incidence in the 3-month toxicity study and the carconogenicity study in mice and in the 3-, 6- and 12-month toxicity studies in dogs with no/low safety margin at a NOAEL compared to the proposed human dose of 20 mg. The findings are likely to be irreversible since the incidence was observed during the recovery in the 3- and 6-month dog studies. In men, there were no clinically significant effects on semen parameters up to 6 months with a clinical dose of 20 mg (#H6D-MC-LVCZ).

2. Vasculitis findings should be interpreted cautiously since the relevance to humans and the pathogenic mechanism of drug-related vascular lesions in animals are poorly understood, and the specific biomarkers are not identified. IC351 treatment increased the incidence of vasculitis in mice, rats and dogs but the effects varied considerably between studies. In a 13-week study in mice, there was hemorrhage in mesenteric lymph nodes in the high dose group (400 mg/kg, approximately 9 times the maximum human exposure of unbound drug). In another 3-month study, there was minimal vasculitis in the high dose group receiving 800 mg/kg (7 times the maximum human exposure; exposure estimates varied between and within studies). In Wistar rats, vasculitis occurred in a number of tissues with slight/minimal severity. In general, the incidence was only slightly higher in treated groups than in controls. These effects were seen at exposures anywhere from 2X (mesenteric phlebitis) to 33X the maximum human exposure. In dogs, findings of arteritis occurred in 1 and 6 month studies. Effects included perivascular inflammation in the lungs, increased incidence of coronary arterial lesions and marked disseminated arteritis. These findings occurred in the absence of elevated heart rate and resulted in the drug being placed on clinical hold. The study pathologist, ——— concluded "the high incidence of arteritis that has been associated with high doses of IC351 in the 6-month dog study, and the predominance of arterial changes in the mid- and high-dose groups in the 1-month study are strongly suggestive of a treatment related change or treatment related exacerbation of the spontaneous polyarteritis". In general, drug effects were seen at exposures between 5 and 16 fold in the 1-month study, and between 29 and 54 times in the 6-month study (as measured by mean AUC of unbound drug) the maximum human exposure. Due to the concerns about vasculitis, the Division requested a 12-month toxicology study in dogs. This study was essentially negative at exposures of 3-33 times the maximum human exposure but there was marked neutropenia/thrombocytopenia indicative of type III immunopathy in two dogs (exposures 14 and 18X human exposure). The high dose dog did have perivasculitis in the circumflex branch of the left coronary artery with clinical signs of fever, anorexia and lethargy. In humans, symptoms of hypersensitivity such as myalgia, infection and back pain were the most frequently reported adverse events associated with IC351. The sponsor concluded that neither back pain nor myalgia was associated with inflammatory or myopathic etiologies based on a clinical study, which measured erythrocyte sedimentation rate and serum creatinine kinase.

In dogs, direct measurements of arterial diameter, vascular resistance or blood flow were not conducted to determine if exaggerated hemodynamic effects were associated with the vasculitis (clinical data indicate that combined treatment with antihypertensive drugs generally reduced the episode of myalgia and backpain). In the safety pharmacology studies, moderate tachycardia (40-60 bpm) was observed in dogs at doses ≥30 mg/kg (1/2 at 30 mg/kg & 2/2 at 100 mg/kg). The effective antihypertensive dose in rats is 1 mg/kg, po. In conscious dogs, single oral doses of 20 and 200 mg/kg produced slight reductions in mean blood pressure without effects on heart rate or respiration rate. In the 1-month study (D20863), the high dose produced a moderate decrease in heart rate with some vasculitis in the lung, spinal cord and thymus. In a 6-month study (D21235), there were no drug-related changes in ECG but there was increased incidence and severity of arteritis with clinical signs in the high dose dogs. It seems that IC351 can induce vascular changes in the absence of any significant effect on cardiac function.

In dogs with drug blood levels below approximately 29 times the human exposure, vascular effects, if any, were minimal to slight. In one 28-day study, D20863, there were positive effects (perivascular inflammation in the lungs in 3/6 dogs vs. 0/6 in controls) at approximately the same exposure as men taking 20 mg. In the 12-month study, exposures at the high dose were up to 33 times the human exposure to free drug with essentially negative effects. It is not known if humans are more or less sensitive to this effect than animals.

Although the data are not particularly convincing that the finding in animals is relevant to humans, hypersensitivity is the major manifestation of clinical drug-induced vasculitis. There were two dogs with symptoms of marked thrombocytopenia (14 and 18X the human exposure) indicative of type III immunopathy in the 12-month dog study and symptoms of back pain and myalgia as the most frequent adverse effects in men. Thus, it would seem to be prudent to include in the label some information on vasculitis in animals.

III. Administrative

A. Reviewer signature: _____

B. Supervisor signature: **Concurrence -** _____

Non-Concurrence - _____
(see memo attached)

C. Cc: NDA 21-368
HFD-580/D. Spell-LeSane, M. Hirsch, A. Batra, G. Benson, M.-J. Ng, D. Hoberman, S. Roy, V. Jarugula, A. Parekh, M. Rhee, R. Agarwal, Y. Shin, A. Jordan
HFD-510/J. El-Hage, K. Davis-Bruno

TABLE OF CONTENTS - PHARMACOLOGY/TOXICOLOGY REVIEW

PHARMACOLOGY/TOXICOLOGY REVIEW

I. PHARMACOLOGY (see Reviews #1/3/4 for IND 54,553

Primary pharmacodynamics:

Mechanism of action: IC351 inhibits PDE5, a major cGMP-hydrolyzing enzyme in human cavernosal smooth muscle, and enhances relaxant effects of NO by stimulating cGMP levels. This leads to relaxation of penile resistance arteries and cavernosal trabecular smooth muscle (#00-0010-11).

Drug activity related to proposed indication: IC351 enhances the erectile response by smooth muscle relaxation and inflow of blood into the penile tissues.

Secondary pharmacodynamics: IC351 had no direct effect on human platelet aggregation but markedly potentiated the anti-aggregatory effect of soluble guanylyl cyclase activator sodium nitroprusside (SNP) in a dose-dependent manner (#97-002-14).

Pharmacology summary: IC351 is a potent inhibitor of PDE5 among 10 PDEs (700- to 49,000-fold) *in vitro* (#00-0006-11). IC351 displays >10,000-fold selectivity for PDE5 against PDE3A found in cardiac myocytes and 700-fold selectivity against PDE6 in photoreceptor. PDE5 was not present in human cardiac myocytes (#00-0009-11). The intermediate derivatives of IC351 (catechol and methylcatechol) and the methylcatechol glucuronide metabolite were 45- and 230-fold less potent than IC351 with some selectivity for PDE5. The major circulating metabolite in human plasma and urine, methylcatechol glucuronide, displays 13,000-fold less potency than IC351 as a PDE5 inhibitor, but does not show selectivity. Sponsor stated that methylcatechol glucuronide would not have clinically significant effects on any of the human PDEs at concentrations achieved in human plasma following efficacious oral doses (#01-0007-11).

Pharmacology conclusions: IC351 strongly potentiated the inhibitory effects of SNP on human platelet aggregation with complete inhibition at 0.25 μM, and increased cGMP levels in the presence of SNP in human cavernosal smooth muscle, suggesting that the PDE5 inhibition by IC351 may lead to large increases in cGMP levels once activated.

II. SAFETY PHARMACOLOGY (see Reviews #1 for IND 54,553

Safety pharmacology summary: IC351 produced emesis/tachycardia at ≥30 mg/kg (p.o.) in conscious dogs (1/2 at 30 mg/kg and 2/2 at 100 mg/kg) and ptosis/depression of the pinnal reflex at 200 mg/kg in rats. Decrease in mean arterial blood pressure was observed at ≥1 mg/kg (p.o.) in hypertensive/normotensive rats and ≥20 mg/kg (p.o.) in conscious dogs. IC351 at cumulative i.v. doses of 0.1 to 3 mg/kg produced dose-dependent decreases in blood pressure in anesthetized dogs secondary to decreased vascular resistance. Administration of IC351 to conscious guinea pigs at an oral dose of 400 mg/kg produced significant reduction in heart rate and progressive bradycardia, concurrent with deteriorating clinical signs and weight loss resulting in death (#S21422). IC351 potentiated ANF-induced diuresis and natriuresis in rats at 0.1 mg/kg (i.v.). IC351 had affinity for the D_2 receptor at 1 μM.

Safety pharmacology conclusions: The effective antihypertensive oral doses of IC351 were 1 mg/kg in the rat and 20 mg/kg in conscious dogs.

III. PHARMACOKINETICS (see Reviews #1/3/4 for IND 54,553

PK parameters: PK of IC351 are linear with respect to time and dose in healthy subjects or in patients with ED. Exposure (AUC) increased proportionally over a dose range of 2.5- to 20 mg. C_{max} was

achieved at a median time of 2 hrs after dosing. Steady-state plasma concentrations were attained by Day 5 with doses of 10- or 20 mg/day with a $t_{1/2}$ of 17.5 hours.

Absorption: Absorption of IC351 is generally rapid in rats and dogs with T_{max} of 1 to 2 hours and 6 hours with oral administration of 10 mg/kg [^{14}C]-IC351, respectively. Repeated oral dosing caused a variable and prolonged T_{max}, suggesting the possibility of absorption in the lower intestinal tract. Oral bioavailability at 10 mg/kg was 34-53% in rats and 10-18% in dogs. Plasma half-life after oral administration in rats and dogs could not be calculated due to the limited number of data points and a prolonged absorptive phase.

Distribution: An oral dose of [^{14}C]IC351 (10 mg/kg) to rats revealed the highest concentrations of radioactivity in the stomach, GI tract, thyroid and lung. By 168 hrs after dosing, radioactive drug-related material was not detected in any tissues except for the liver. Exposure to pregnant rats on gestation Day 18 caused the highest concentrations of radioactivity in maternal adrenal gland, preputial gland and liver at 8 hours post-dose. Parent and/or metabolites of IC351 were detected in the maternal placenta, and fetal adrenal gland, blood, brain, eye, kidney, liver & myocardium with substantially low exposure at 8 hours post-dose, indicating the placental transfer.

Metabolism: IC351 is predominantly metabolized by CYP3A4 in human liver. Unchanged IC351 accounted for 26% in human plasma, indicating an extensive metabolism. In human feces, the majority of the radioactivity was associated with metabolites after 24 hours. In human, dog and mouse liver slices, the major metabolite was the methylcatechol glucuronide. Catechol glucuronide was the most abundant metabolite in rat liver.

Excretion: Major route of elimination of a radiolabled dose (100 mg) was the feces with 61% and urine with 36% in human by 13 days. Elimination of radioactivity in feces at a single dose of 10 mg/kg was 98% in rats, 84% in male dogs and 63% in female dogs. Only ≤0.1% of administered IC351 is excreted in maternal milk over a 3 to 24 hour period, suggesting that maternal milk is not a major route of elimination for IC351 and metabolites.

Protein binding: *In vitro* plasma protein binding to mouse, rat, dog and human was determined to be 85%, 92%, 87% and 94%, respectively.

PK/TK summary: Plasma concentrations generally increased sub-proportionally to the increased dose in mice, rats and dogs. Exposure was higher after 1 to 3 months in rats and dogs, suggesting accumulation in the plasma. The plasma exposure in pregnant rats following daily oral dosing from gestation Days 6 through 12 was also less than proportional to dose over the range of 60 to 1000 mg/kg with T_{max} of 4 to 12 hours. The only gender difference was a higher exposure in female rats, and is not marked in humans.

Summary of $AUC_{(0\text{-}24h)}$ in Subchronic- and Chronic Studies for Mice, Rats and Dogs

Species	Study #	Duration		Dose, mg/kg	$AUC_{(0\text{-}24h)}$, ng·h/mL	
					M	F
Mouse (CD-1)	CTBR88440	3 Months	Day 90	60	18231	13390
				200	19701	26275
				400	26860	27152
	M04398	3 Months	Day 90	60	7886[a]	13492[a]
				200	17822[a]	22699[a]
				400	18559[a]	19827[a]
				800	20004[a]	22421[b]
	CTBR88780	6 Months (Carcinogenicity)	Day 180	10	7125[c]	7023[c]
				60	14999[d]	12062[d]

				400	31223[d]	20962[d]
Rat (Wistar)	R20861	1 Month	Day 27	10	13280[e]	25010[e]
				60	54120[e]	74320[e]
				400	83910[e]	159600[e]
	R18498 (Fisher)	3 Months	Day 90	60	29427	46354
				100	21221	51802
				400	49951	126171
				800	41649	77182
	R21236	6 Months	Day 168/169	10	14900[e]	28200[e]
				60	29100[e]	82900[e]
				400	72200[e]	190000[e]
	CTBR88779	6 Months (carcinogenicity)	Day 180	10	16070	35899
				60	38604	91106
				180	78863	152863
Dog (beagle)	D20863	1 Month	Day 28	10	5060-11100	2390-4160
				45	6750-7420	15300-33200
				200	209000-230000	131000-138000
	88270	3 Months	Day 91	10	2565-14553	-
				60	11907-21669	-
				200	35629-74920	-
	CTBR88632	6 Months	Day 176	10	NC-6882	NC-6202
				60	13370-25863	11424-44885
				200	13007-62645	16983-55001
				400	31384-91270	41786-129341
	D21235	6 Months	Day 182/183	10	4350-43200	4960-26900
				60	13100-119000	36600-98600
				400	68300-179000	44300-261000
	D01899	1 Year	Day 364	25	8576-43136	8792-68012
				100	12122-62109	39276-158317
				400	16900-77350	28936-109961
Human	LVDK		Day 5	20 mg	7692	-

[a]$AUC_{(0\text{-}12h)}$, [b]$AUC_{(0\text{-}16h)}$, [c]$AUC_{(0.5\text{-}\tau)}$, [d]$AUC_{(0\text{-}\tau)}$, [e]$AUC_{(1\text{-}24h)}$

PK/TK conclusions: Metabolism was the primary mechanism for clearance of IC351 from the systemic circulation with similar routes of biotransformation in mice, rats, dogs and humans. The major route of excretion was via the feces in both rats and dogs, indicating incomplete oral absorption and biliary excretion of metabolites. Slight- to moderate increases in hepatic enzyme activity and/or CYP450 content were observed in mice, rats and dogs after oral doses of ≥400 mg/kg, indicating IC351 as a inducer of CYP450 isoenzymes.

IV. TOXICOLOGY (see Reviews #1/2/3 for IND 54,553

Study title: 1-Year Oral Toxicity Study in Beagle Dogs

Key study findings: Testicular degeneration/atrophy was observed with deceased sperm in the epididymides at all treatment groups with increased severity following 12 months. Neutropenia, thrombocytopenia and/or anemia were observed in the mid- and high dose females (1/5 each group).

Study no: D01899 **Volume #, and page #:** vol. 31
Conducting laboratory: Eli Lilly & Company, Greenfield, IN 46140
Date of study initiation: June 19, 1999 **GLP compliance:** Yes
QA report: yes (x) no ( )
Drug/lot #: IC351 (LY450190)/991020 **% purity:** 100.1%
Formulation/vehicle: Gelatin capsule/1% (w/v) carboxymethylcellulose sodium & 0.5% sodium lauryl sulfate in purified water

Dosing:

Species/strain: Beagle dog
#/sex/group or time point (main study): 5/sex/group
Satellite groups used for toxicokinetics or recovery: no recovery studied
Weight: 9.0 to 12.9 kg for males & 7.3 to 9.9 kg for females **Age:** 14 to 15 months

Doses in administered units: 0, 25, 100 & 400 mg/kg/day
Route, form, volume, and infusion rate: Oral suspension of 0, 12.5, 50.0 & 200.0 mg/mL in capsules

Observations and times:

Observations	Times
Mortality/Moribundity Clinical Signs	More than once daily
Body Weights/Food Consumption	Twice daily pre-study phase/Once weekly post-dose phase
Ophthalmoscopy	Pretreatment & terminal examination
Pathology/Urinalysis/Organs Weighs	Scheduled necropsy on Days 365 & 366
Electrocardiography	Day -7/pre-dose & 2 hrs post-dose on Days 180 & 362
Clinical Pathology	Twice daily pre-study phase/Months 1, 3, 6, 9 & 12 for fasted Months 5, 8, 10, 11 & 12 for non-fasted
Toxicokinetics	0, 1, 2, 4, 8, 12, 16 & 24 hrs on Days 0, 33, 177 & 364

Results: Treatment was suspended for one 100- & one 400 mg/kg female dogs between Days 140 and 166 & from Days 196 through the end of the study due to marked neutropenia (1,610/µL for 100 mg/kg & 310/µL for 400 mg/kg compared to reference 3,300 to 11,600/µL) with moderate thrombocytopenia (90,000/µL for 100 mg/kg & 226,000/µL for 400 mg/kg compared to reference levels of 191,000 to 442,000/µL), which was initially identified on Day 91. Severe neutropenia (170/µL), moderately decreased platelets (111,000/µL), hyperglobulinemia, minimal anemia, minimal eosinopenia and ↑ALP in the absence of clinical signs were present in the 100 mg/kg dog on Day 140. The other blood dyscrasias included minimal monocytopenia, lymphopenia & eosinopenia in both dogs, which the sponsor considered to be due to stress. Clinical signs of fever (105.4°F), anorexia & lethargy followed by minimal neutropenia (2,930/µL), anemia, severe monocytosis & slight hyperglobulinemia compatible with inflammation occurred in the 400 mg/kg dog on Day 128. Abdominal radiographs taken on Days 128 & 288 excluded an occult inflammatory focus or splenic enlargement. Antibiotic therapy was initiated on Day 128 due to signs of inflammation (↑basophilia/Dōhle bodies). After removal from antibiotics on Day 138, clinical signs returned to normal with severe neutropenia (200/µL) & neutrophil alterations. The dog became febrile & developed neck pain/inappetence on Day 141 despite the reinstatement of antibiotic on Day 139. Additional aspirin & antibiotics were administered on Days 146 through 154. Following drug removal (& antibiotic & supportive therapy in the 400 mg/kg) on Days 140 (400 mg/kg) & 142 (100 mg/kg), dosing resumed on Days 161 (400 mg/kg) & 166 (100 mg/kg). Marked neutropenia (1,130/µL for 100 mg/kg & 1,210/µL for 400 mg/kg) and/or minimal thrombocytopenia developed within 8 days in the 400 mg/kg or within 10 days in the 100 mg/kg dog. These dogs were clinically asymptomatic until Days 195 when the 400 mg/kg dog developed clinical signs of stiffness/neck pain/anorexia, neutrophilia (10,250/µL), monocytosis & hyperglobulinemia. Discontinuation of IC351 on Day 196 returned the neutrophil (Day 204 for 100 mg/kg & Dat 196 for 400 mg/kg) and/or platelet counts (Day 208 for 100 mg/kg) within the reference interval & without clinical signs. Bone marrow aspirates and core biopsies from humerus taken from these 2 dogs on Day 196 demonstrated no abnormalities but increased numbers of immature neutrophilic precursors and myeloid/megakaryocytic hyperplasia, which were less pronounced on Days 231 (100 mg/kg) or 285 (400 mg/kg) and absent on Day 366. Sponsor considered the findings to be idiosyncratic and not a result of a direct compound-related effect on early neutrophil precursors or on bone marrow. The high-dose female had a single focus of perivasculitis in the circumflex branch of the left coronary artery. The 100 mg/kg dog showed a pituitary neuroblastoma, which was considered to be spontaneous.

Drug-related degeneration & atrophy of the seminiferous epithelium occurred in dogs at ≥25 mg/kg with decreases in testicular weight & gross findings of small/soft testes. Degeneration of the seminiferous

epithelium was characterized by disassociation or vacuolation of seminiferous epithelium, mutinucleated cells, exfoliated germ cells, pyknotic spermatids, megalospermatids, attenuation or loss of cell layers, and/or increase in Leydig cells. Low numbers of tubules also had markedly enlarged cells of spermatogonia with abundant eosinophilic cytoplasm & round nuclei with rarefaction of the chromatin. Scattered tubules had individual cell necrosis of the mitotic spermatocytes and/or elongating spermatids. Atrophy of the seminiferous epithelium was characterized by seminiferous tubules lined by only Sertoli cells and/or spermatogonia. Degeneration and atrophy were more pronounced in the periphery of the affected testes. These testes were decreased overall in diameter with obliteration of the lumen or attenuation of the epithelium with increased intraluminal space compared to controls. Aspermia were also noted, which was considered secondary to decreased testicular sperm production.

Dose, mg/kg, n=5	0	25	100	400
Testes weight (g), absolute	12.68	11.38	10.00	8.22*
Epididymides weight (g), absolute	3.00	3.10	2.60	2.68
Testes, small		1	2	4
Testicular seminiferous epithelium				
Bilateral degeneration	0	2 minimal 2 slight 1 severe	3 slight 2 moderate	1 slight 1 moderate 3 severe
Bilateral atrophy	0	1 marked	1 minimal 2 slight	1 marked 2 severe
Epididymis				
Bilateral aspermia	0	1	0	2
Bilateral multifocal epithelial vacuolation	1 minimal	1 minimal	2 minimal	2 slight

*Significantly different from control at $p \leq 0.05$

Table below summarizes the results for the rest of the animals except the 2 female dogs with marked neutropenia. No drug-related arteritis lesions occurred in the present study, due perhaps to lower blood drug levels or different dog colony. No drug-related mortality was observed. Abnormal feces were more frequently observed in all treated groups. There was dose-dependent decrease in mean body weight in females, which was observed by 6 weeks after study initiation, & maintained without additional changes. WBC parameters of reticulocytes, neutrophils and monocytes were decreased from mid dose. IC351 produced slight dose-dependent increases in CYP450 content in males with increased liver weight. Increased adrenal weight was observed with enlarged/mutifocal agonal hemorrhage and multifocal accessory structure in a high dose male dog. Slightly increased incidence of hepatic leukocytosis/centrilobular pigmentation was found in the high dose group. Sponsor considered these & all other findings spurious due to the small magnitude of the change, individual variablity & overlap with concurrent controls. Plasma exposure increased on repeated dosing with marked variability within the same treatment group. Half-life was not calculated due to the limited number of time points. TK data were taken directly from the submission.

Dose, mg/kg	0		25		100		400	
	5M	5F	5M	5F	5M	4F	5M	4F
Mortality	0	0	0	0	0	0	0	0
Clinical signs, n=5								
Feces, pale	0	0	4	3	5	5	5	5
Lameness	0	0	0	0	0	0	2	0
Skin, laceration/abrasion/red	0	0	0	0	0	0	1	0
Skin, swollen	0	0	1	0	0	0	1	0
Digit, swollen	0	0	1	0	0	0	1	0
Decreased activity	0	0	0	0	0	0	0	1
Vaginal discharge, brown	na	0	na	0	na	0	na	1
Mammary gland, swollen	0	0	0	1	0	1	0	1

Body weights, kg, Day 364	12.36	11.30	12.58	**10.48**	12.28	**9.40**	12.54	**9.55***
Food consumption	UR	UR	UR	UR	UR	UR	UR	UR
Ophthalmoscopy	UR	UR	UR	UR	UR	UR	UR	UR
Hematology, Day 363								
Reticulocytes, $10^3/\mu L$	68.4	80.6	83.4	105.6	73.0	**56.5**	**43.0**	**42.8****
Neutrophils, $10^3/\mu L$	7.036	7.694	6.290	5.174	6.804	5.598	**5.812**	**6.305**
Monocytes, $10^3/\mu L$	0.578	0.668	0.482	**0.340*****	0.568	**0.450***	**0.466**	**0.610****
Clinical chemistry, Day 363								
Cholesterol, mg/dL	150.84	206.7	163.86	225.84	156.02	190.90	**183.70**	202.05
Triglyceride, mg/dL	27.66	53.60	34.24	37.12	29.38	31.88	**43.82**	35.95
Inorganic phosphorus, mg/dL	4.020	3.820	3.480	3.700	3.540	3.075**	3.380	3.525*
Electrocardiography	UR	UR	UR	UR	UR	UR	UR	UR
Urinalysis	UR	UR	UR	UR	UR	UR	UR	UR
Hepatic Microsomal Enzyme,								
Total CYP450, nmol/mg protein	0.389	0.315	**0.411**	0.433	**0.459**	0.426	**0.508***	**0.418**
Organ weights, absolute, g								
Kidneys	53.26	43.90	48.74	37.18*	49.26	**33.23****	53.92	**36.10***
Liver	248.4	298.0	318.2	238.8	**276.4**	228.8	**318.8**	250.5
Adrenals	1.346	1.240	1.427	1.387	1.441	1.380	1.615	1.260
Thyroids	0.678	0.616	0.818	0.643	0.761	0.597	0.861	0.759
Gross pathology,								
Alopecia	0	0	0	0	0	0	0	1
Stomach, lesion	0	0	0	0	0	0	0	1
Liver, lesion	0	0	0	2	1	0	1	0
Spleen, lesion	0	0	0	1	0	1	1	0
Lymph node, enlarged	0	0	0	0	0	0	0	1
Skin, lesion	0	0	0	0	0	0	0	1
Adrenal, enlarged	0	0	0	1	0	1	1	0
Thyroid, enlarged	0	0	0	0	1	0	1	1
Pituitary, enlarged	0	0	0	0	0	0	1	0
small	0	0	0	0	0	0	1	0
Histopathology,								
Liver, diffuse sinusoidal leukocytosis	2	0	3	0	1	0	3	2
subacute multifocal perivasculitis	3	3	1	2	3	3	5	2
centrilobular hepatocellular pigmentation	1	0	1	1	0	1	3	1
Gallbladder, multifocal lymphocytic infiltration	0	0	1	0	1	3	0	1
Lung, chronic focal proliferative bronchiolitis	0	0	0	1	0	0	1	0
chronic multifocal proliferative bronchiolitis	2	2	1	0	2	1	3	1
Spleen, hemosiderosis	0	0	1	0	0	0	1	0
Salivary gland, multifocal lymphocytic infiltration	0	0	1	0	0	0	1	0
Tongue, multifocal lymphocytic infiltration	0	0	0	0	0	0	1	0
Prostate, multifocal atrophy	1	na	4	na	2	na	2	na
multifocal acinar dilation	2	na	4	na	5	na	3	na
multifocal fibrosis	0	na	1	na	1	na	1	na
chronic focal inflammation	0	na	1	na	1	na	1	na
Skeletal muscle, multifocal degeneration	0	0	0	0	0	0	1	0
Adrenal, multifocal cortical accessory structure	0	1	0	0	1	0	1	0
Thyroid, multifocal mineralization	0	0	0	0	0	0	1	0
Parathyroid, focal ductal cyst	0	0	0	0	0	0	0	1
Pituitary congestion	0	0	0	0	0	0	1	0
Cerebrum, acute multifocal agonal hemorrhage	0	0	0	0	0	0	1	0
Brain stem, acute multifocal hemorrhage	0	0	0	0	0	0	1	0

Significantly different from control at $p \le 0.05^*$, $p \le 0.01^{**}$ or $p \le 0.001^{***}$
UR- unremarkable

Compound: IC351 (LY450190)
Study: D01899

Table 1: Summary of Plasma Exposure Parameters in Beagle Dogs after Oral Administration of 25, 100, or 400 mg IC351/kg/day for Up to 1 Year

Parameter	Administered Dose (mg/kg/day)					
	25		100		400	
Sex	Male [a]	Female [a]	Male [a]	Female [b]	Male [a]	Female [b]
Day 0						
Range of $AUC_{0\text{-}24\ hr}$ (ng•hr/mL)						
Mean (± SD) C_{max} (ng/mL)	655 ± 499	578 ± 143	708 ± 264	1196 ± 1216	2662 ± 2251	4058 ± 2729
T_{max} (hr)	2 to 16	2 to 4	2 to 12	2 to 24	2 to 24	4 to 16
Day 33						
Range of $AUC_{0\text{-}24\ hr}$ (ng•hr/mL)						
Mean (± SD) C_{max} (ng/mL)	737 ± 462	1159 ± 349	1201 ± 565	4255 ± 2233	3159 ± 1862	4168 ± 2168
T_{max} (hr)	2 to 12	2 to 8	2 to 8	0 to 24	2 to 8	2 to 4
Day 177[c]						
Range of $AUC_{1\text{-}24\ hr}$ (ng•hr/mL)						
Mean (± SD) C_{max} (ng/mL)	1123 ± 584	1987 ± 1026	1758 ± 928	3690 ± 906	3164 ± 1599	5914 ± 2354
T_{max} (hr)	2 to 16	2 to 12	2 to 8	1 to 16	4 to 12	2 to 12
Day 364						
Range of $AUC_{0\text{-}24\ hr}$ (ng•hr/mL)						
Mean (± SD) C_{max} (ng/mL)	1440 ± 706	2301 ± 1356	2706 ± 1748	4763 ± 2660	3207 ± 1820	5413 ± 2512
T_{max} (hr)	2 to 8	2 to 4	4 to 12	8 to 24	4 to 8	12 to 16

[a]N = 5 dogs/sex; [b]N= 4 dogs/sex as Dog 283863 (100 mg/kg) and Dog 284504 (400 mg/kg) were excluded from calculation of summary statistics as a result of cessation of dosing on multiple periods, for extended lengths of time; ; [c]Time zero plasma sample not collected – AUC calculated from 1 to 24 hours.

Abbreviations: AUC = Area under the plasma concentration-time curve from 0 to 24 hours; C_{max} = maximal observed plasma concentration; SD = standard deviation; T_{max} = range of time to reach C_{max}.

Human $AUC_{0\text{-}24}$ at steady state= 7,700 ng•hr/mL with 20 mg/day (LVDK)

IND No.

Summary: No NOAEL was identified due to testicular findings. Present study was conducted using a CMC/[redacted] instead of IC351: [redacted] used in the previous studies based on a pilot study (Study #D02799; data not provided) as it yielded the most consistent plasma levels and is comparable to the market image according to the sponsor. Plasma exposure to IC351 increased sub-proportionally to the dose with marked variability within the same treatment group possibly due to absorption in the lower intestine. No drug-related mortality was observed. Mean body weight for females in the 100- and 400 mg/kg groups was decreased by 6 weeks after dosing which persisted throughout the study. Increase in weight & histopathological findings in the liver correlated with an increase in total hepatic CYP450. Incidence of small/soft testes and testicular degeneration/atrophy of seminiferous epithelium at all dose groups correlated with concomitant decrease in testis weight and aspermia in epididymides. The incidence tended to be more severe compared to 6-month studies, suggesting that severity increases with chronic dosing. Although the reversibility of the finding is unknown for the 1-year study, extensive cell loss in the germinal epithelium was observed in dogs with the most severe testicular alterations, such that reversibility is unlikely. Sponsor indicated that morphologic alterations such as necrosis of spermatogonia, and Leydig cell hyperplasia and prostatic atrophy, which are suggestive of direct cytotoxicity and disruption of the pituitary-gonadal hormonal axis, respectively, were not observed.

Marked neutropenia & thrombocytopenia were observed in one female dog of the mid- and high dose groups, which was reversible within 2 weeks after removal from the drug. The 400 mg/kg female exhibited anemia, neutropenia, thrombocytopenia & perivasculitis in the circumflex branch of the left coronary artery. The effects in the 400 mg/kg female were accompanied by clinical signs of fever, anorexia & lethargy. Bone marrow samples and sera taken from these 2 dogs on Day 196 demonstrated no detectable anti-RBC antibodies with a modified indirect Coomb's test, but did show an increase in neutrophilic precursors and myeloid/megakaryocytic hyperplasia. Sponsor considered that these findings were idiosyncratic, and not a result of a direct effect on bone marrow hematopoietic precursors. The sponsor also ruled out some potential mechanisms of drug-induced hematologic disorders since (1) there was a relatively short recovery time and localization of effects to the mature neutrophil populations, indicating no direct effects on immature neutrophilic precursors and/or bone marrow stem cells; (2) there were no clinical signs, inflammatory leukograms & neutrophil cytoplasmic changes in the 100 mg/kg dog, suggesting neutrophil consumption with inflammation or sequestration was not responsible for the neutropenia; (3) inflammatory changes (neutrophilia/monocytosis/neutrophil cytoplasmic changes and/or hyperglobulinemia) in the 400 mg/kg dog were limited to periods when the dog exhibited clinical signs; (4) antibiotic & supportive therapy alone failed to resolve neutropenia; and (5) there was no evidence of splenic enlargement, commonly associated with peripheral consumption of blood cells.

TOXICOLOGY SUMMARY AND CONCLUSIONS:

PDE inhibitors are associated with disseminated arteritis and testicular degeneration in dogs and in other animal species. IC351 also caused seminiferous epithelial atrophy of the testis in a dose-dependent manner, which correlated with a decrease in testicular weight and oligo/aspermia in the dog studies. These findings were observed in the 3-month toxicity study and the carcinogenicity study in mice and in the 3-, 6- and 12-month toxicity studies in dogs with low multiple of exposure compared to the human exposure at a dose of 20 mg. The lesions are likely to be non-reversible in dogs, and the severity increased with chronic dosing. There were no clinically significant effects on semen parameters up to 6 months with a dose of 20 mg in 217 men (#H6D-MC-LVCZ).

In dogs, arteritis was observed in multiple tissues including thymus, lung, and spinal cord at high doses with increased incidence from the 1-month study. Coronary arteritis was observed at ≥45 mg/kg in the 1-month study with no hemodynamic effects (vasodilation/tachycardia), suggesting a drug-related effect. In the 3-month study, myocardial degeneration, fibrosis and epicarditis was observed at 200 mg/kg

in a single male dog (1/4). The 6-month study showed slight increased incidence and severity of disseminated periarteritis at 400 mg/kg in multiple tissues including coronary arteries. The periarteritis was associated with medial/epicardial/subendothelial inflammatory cell infiltration, neutrophilic adventitial inflammation and medial fibrinoid necrosis. The one-year dog study did not duplicate the vasculitis findings observed in the previous dog studies. The reason may be due in part to different dog colony or lower drug exposures. One high dose female dog exhibited a single focal perivasculitis in the circumflex branch of the left coronary artery and marked neutropenia/thrombocytopenia, anemia and hyperglobulinemia with fever, lethargy and anorexia. Another female in the mid-dose group developed moderate neutropenia/thrombocytopenia, and treatment for both dogs was suspended during the study. No other lesions for drug-induced vasculitis of atrial epicardial hemorrhage or myocardial necrosis were observed. Sponsor considered that the findings for the 2 dogs were drug-related idiosyncratic hematologic disorders. The NOAEL for the vasculitis findings produced approximately 1- to 3-fold exposure multiples for 1-month study at 10 mg/kg and 3- to 33-fold exposure multiples for 6-month study at 60 mg/kg of unbound parent drug (due to individual variability) to humans taking 20 mg. Periarteritis and hemorrhage/necrosis of lymphoid were also observed in mice and rats with moderate safety margins (<10 fold) at the NOAEL.

Daily oral administration of IC351 to rats for 6 months was generally well tolerated up to 400 mg/kg without drug-related effects on mortality or body weight. Brown pigment deposition in the cytoplasm of periportal hepatocytes associated with focal accumulation of Kupffer cells was observed with increased liver weight in female rats given 60 and 400 mg/kg. The hepatocellular pigmentation was also observed in male dogs of the 3- and 12-month studies at 200 and 400 mg/kg, respectively. Phlebitis of mesenteric beds at all treated groups and hepatic arteritis in high dose rats were noted. Other histopathological findings included renal tubular epithelial regeneration/hyperplasia, splenic extramedullary hematopoiesis or hemorrhage in the lymph node/thymus with increased frequency in the high dose group.

IC351 administered at doses up to 800 mg/kg/day for 3 months in mice produced no treatment-related deaths or body weight changes. Periarteritis in the testicular/mesenteric arteries was observed with lymphocytic atrophy/necrosis in the spleen/thymus of the high dose group. These lesions were correlated with epididymal bilateral epithelial vacuolation or prostatic chronic focal inflammation. Other microscopic findings included splenic hematopoiesis in all treated groups.

Labeling Recommendations under Animal Toxicology:

Histopathology Inventory for NDA #21-368

Study	1-mo	1-mo	3-mo	3-mo	3-mo	6-mo	6-mo	1-yr	2-yr	2-yr
Species	Rat	Dog	Mouse	Rat	Dog	Rat	Dog	Dog	Mouse	Rat
Adrenals	X*	X*	X*	X*	X*	X*	X*	X*	X	X
Aorta	X	X	X	X	X	X	X	X	X	X
Bone Marrow smear	X	X	X	X	X	X	X	X	X	X
Bone (femur)	X	X				X	X	X		
Brain	X*	X*	X*	X*	X*	X*	X*	X*	X	X
Cecum	X	X	X	X	X	X	X	X	X	X
Cervix			X	X				X		
Colon	X	X	X	X	X	X	X	X	X	X
Duodenum	X	X	X	X	X	X	X	X	X	X
Epididymis	X	X	X	X	X	X	X*	X*	X	X
Esophagus	X	X	X	X	X	X	X	X	X	X
Eye	X	X	X	X	X	X	X	X	X	X
Fallopian tube										
Gall bladder		X	X		X		X	X	X	
Gross lesions	X	X	X	X	X	X	X	X	X	X
Harderian gland	X		X	X		X			X	X
Heart	X*	X*	X*	X*	X*	X*	X*	X*	X	X
Ileum	X	X	X	X	X	X	X	X	X	X
Injection site										
Jejunum	X	X	X	X	X	X	X	X	X	X
Kidneys	X*	X*	X*	X*	X*	X*	X*	X*	X	X
Lachrymal gland		X	X		X		X	X	X	X
Larynx	X	X	X	X	X	X	X			
Liver	X*	X*	X*	X*	X*	X*	X*	X*	X	X
Lungs	X*	X*	X*	X	X	X*	X	X	X	X
Lymph nodes, cervical	X	X		X	X	X	X	X		
Lymph nodes mandibular			X				X	X	X	X
Lymph nodes, mesenteric	X	X	X	X	X	X	X	X	X	X
Mammary Gland	X	X	X	X	X	X	X	X	X	X
Nasal cavity										
Optic nerves	X	X	X		X	X	X		X	X
Ovaries	X*	X*	X*	X*		X*	X*	X*	X	X
Pancreas	X	X	X	X	X	X	X	X	X	X
Parathyroid	X	X*	X*	X*	X*	X	X*	X*	X	X
Peripheral nerve	X	X	X	X		X				
Pituitary	X*	X*	X*	X*	X*	X*	X*	X*	X	X
Prostate	X*	X*	X*	X*	X*	X*	X*	X*	X	X
Rectum	X	X	X	X	X	X	X	X	X	X
Salivary gland	X	X	X	X	X	X	X	X	X	X
Sciatic nerve			X		X			X	X	X
Seminal vesicles	X		X	X		X			X	X
Skeletal muscle	X	X	X	X	X	X	X	X	X	X
Skin	X	X	X	X	X	X	X	X	X	X
Spinal cord	X	X	X	X	X	X	X	X	X	X
Spleen	X*	X*	X*	X*	X*	X*	X*	X	X	X
Sternum	X		X	X	X	X	X			
Stomach	X	X	X	X	X	X	X	X	X	X
Testes	X*	X*	X*	X*	X*	X	X*	X*	X	X
Thymus	X*	X*	X*	X*	X*	X*	X*	X	X	X
Thyroid	X	X*	X*	X*	X*	X	X*	X*	X	X
Tongue	X	X	X	X	X	X	X	X	X	X
Trachea	X	X	X	X	X	X	X	X	X	X
Urinary bladder	X	X	X	X	X	X	X	X	X	X
Uterus	X	X	X*	X	X	X	X*	X	X	X
Vagina	X	X	X	X	X	X	X	X	X	X
Zymbal gland	X					X				
Hepatic artery			X	X		X	X	X		
Mesenteric artery & vein			X	X		X	X	X		
Testicular artery			X	X		X	X	X		
Coronary artery		X					X	X		

X, histopathology performed
*, organ weight obtained

V. GENETIC TOXICOLOGY (see Reviews #1 for IND 54,553

IC351 was not genotoxic or mutagenic in *in vitro* bacterial Ames test, mammalian cell mutation assay or cytogenetic study in human lymphocytes, and not clastogenic in *in vivo* rat micronucleus assay.

Labeling recommendations:

VI. CARCINOGENICITY:

Study title: 2-Year Oral Gavage Carcinogenicity Study of IC351 in Albino Mice

Key study findings: Degeneration/atrophy of testicular tubular epithelium associated with oligo/aspermia in the epididymis was slightly increased at ≥60 mg/kg. Hepatocellular adenomas and alveolar/bronchiolar adenomas/carcinomas increased in the high dose males and females, respectively, but was not statistically significant. The AUC of the high dose (males and females) for the unbound parent drug was approximately 10 times the human AUC at the proposed clinical dose of 20 mg.

Study number: 88455 — 88780 for — **Volume #, and page #:** vol. 33
Conducting laboratory and location:
Date of study initiation: December 19, 1997
GLP compliance: yes **QA report:** yes (x) no ( )
Drug: IC351 (LY450190,) **Lot # (% purity):** F96/048A (47.0%), 43582 (47.1%)
CAC concurrence: Dose selection was not reviewed by the Executive CAC but the committee concurred on the ongoing studies on 6/16/99 based on AUC ratios with a 10 mg human dose (see Appendix II for report).

Study Type: 2-year rodent bioassay
Species/strain: Crl:CD^{R}-1(ICR) mice (*Mus musculus*) **Number/sex/group:** 50/sex/group
Age at start of study: 6 weeks (24.3-32.8 g for males and 19.2 to 25.6 g for females)
Animal housing: Individual
Formulation/vehicle: 0.5% hydroxypropyl methylcellulose (HPMC) containing 1% Tween 80
Drug purity/stability/homogeneity: Accessed
Methods:
Doses: 0, 10, 60 & 400 mg/kg **Basis of dose selection:** AUC ratios
Route of administration: Oral gavage **Frequency of drug administration:** Daily
Dual controls employed: Dual identical controls **Interim sacrifices:**
Satellite PK or special study group(s): 3/sex/timepoint for PK
Deviations from original study protocol: N/A
Statistical methods: Proc Multtest implemented with the Peto's survival-adjusted one-sided trend test
Observations and times:

Observations	Times
Mortality/Clinical Signs	Twice daily/Detailed physical exam weekly
Body Weights/Food Intake	Weekly
Food Consumption	Weekly/Monthly after 13 weeks
Ophthalmology	Weeks -1, 52 & 104
Pathology/Clinical Chemistry	Week 104
Toxicokinetics	Days 21, 84 & 180 at 0, 0.5, 1, 2, 4, 8, 16 & 24 hrs post-dose

RESULTS: Mortality (~50%) was similar in the control and treated groups at the end of the study, although the males in the vehicle group 1 had slightly higher mortality rate from 56- to 100 weeks (see Appendix III for graphical presentation). Major cause of death was urinary tract disorders (inflammation/retention) accounting for 35% in males and lymphoreticular neoplasia in 34% of preterminal euthanized females. There were no treatment-related differences in group mean body weight or food consumption in all groups, and occasional statistically significant differences were not considered to be of biological significance (see Appendix IV for graphical presentation). Clinical signs

included fur staining, scabbing/reddening of the skin and ocular opacities in addition to the decreased activity, dehydration, prominent backbone and masses observed in sacrificed mice, and were considered unrelated to treatment. Plasma exposure was less than proportional to the dose. There were no consistent gender-related differences in the exposure. The half-life could not be calculated due to the limited number of timepoints in the log-linear phase of the plasma concentration versus time curve.

Dose, mg/kg	**0**		**0**		**10**		**60**		**400**	
	50M	50F	50M	50F	50M	50F	50M	50F	50M	50F
Mortality, week 104	25	23	24	25	23	21	23	25	20	26
Clinical Signs,										
Fur thin cover, limbs/paws	4	8	6	8	5	9	10	8	9	6
Fur ungroomed	17	11	3	14	9	11	17	19	15	18
Abnormal breathing, labored/shallow	9	9	9	10	7	6	8	6	15	16
Hunched posture	5	5	5	5	2	1	3	3	9	4
Abnormal feces, decreased/absent	9	5	7	4	4	7	5	6	6	9
Body Weights	UR	UR	UR	UR	UR	UR	UR	UR	UR	UR
Food Consumption	UR	UR	UR	UR	UR	UR	UR	UR	UR	UR
Hematology	UR	UR	UR	UR	UR	UR	UR	UR	UR	UR
Ophthalmoscopy	UR	UR	UR	UR	UR	UR	UR	UR	UR	UR
Gross pathology	See below		See below		See below		See below		See below	
Toxicokinetics, 3/sex/timepoint										
AUC_{0-t} (ng•hr/mL) Day 21					6044*	6776*	19773	18131	29250	32790
Day 84#					5361$	5697	14232	22039	34023	32501
Day 180					7125*	7023*	14999	12062	31223	20962
C_{max} (ng/mL) Day 21					1584	2404	3379	3246	4617	5259
Day 84#					1264	1175	2306	2965	4324	4452
Day 180					1164	1158	1943	2197	5372	3653
T_{max} (hr) Day 21					1.0	2.0	2.0	2.0	1.0	4.0
Day 84#					1.0	1.0	4.0	1.0	4.0	4.0
Day 180					2.0	2.0	1.0	2.0	1.0	2.0

#Blood collection schedule was limited on Day 84 & only partial exposure profiles were obtained.
*$AUC_{0.5-t}$ due to no quantifiable concentration at time 0.
$$AUC_{1.0-t}$ due to no quantifiable concentration at time 0.
UR- unremarkable
Human AUC_{0-24} at steady state= 7,700 ng•hr/mL at 20 mg/day

Non-neoplastic findings: Sponsor stated that there were no treatment-related non-neoplastic findings (see Appendix V for incidence of histopathology findings). However, increased episode of penis protrusion in the high dose group was consistent with inflammation in urinary tract/prostate/seminal vesicle and/or urinary retention, which were the major cause of death and preterminal euthanasia accounting for 35% in males. Gross lesion of soft testes was also associated with atrophy of the testicular tubular epithelium and epididymal oligo/aspermia at ≥60 mg/kg. Increased frequency was observed for the eye opacity in the mid- and high dose males and corneal mineralization/erosion/ulceration in the high dose females. Dark areas and foci in the cecum were more frequently found at high dose males. Edema of the cecum/rectum was observed with increased incidence in the high dose females, which paralleled the amyloidosis in the digestive tract. Histopathological findings in the hematopoietic system and lymphoid were generally associated with various inflammatory process such as dermatitis, cystitis or pyelonephritis. Extramedullary hemopoiesis observed in the liver, adrenal glands, and lymph nodes also paralleled the myeloid hypercellularity in the bone marrow and hemopoiesis in the spleen. Table below summarizes the microscopic findings with increased incidence.

Incidence of Non-neoplastic Findings in Albino Mice

Dose, mg/kg	0		0		10		60		400	
	50M	50F	50M	50F	50M	50F	50M	50F	50M	50F
Bulbopenis, hemorrhage	0	na	5	na	4	na	6	na	9	na
inflammation	0	na	1	na	0	na	2	na	5	na
Cecum, deposits, pigment	5	1	10	4	5	3	6	1	13	6
edema	3	1	1	5	0	6	2	3	0	9
Adrenal, angiectasis	0	0	0	0	0	0	0	0	1	1
Epididymis, oligo/aspermia	20	na	17	na	17	na	23	na	24	na
Eye, erosion/ulceration, cornea	0	4	0	1	3	4	2	6	1	8
Kidney, necrosis, papilla	0	1	1	0	0	0	1	0	0	2
infiltration, mixed cell	1	0	4	0	1	1	2	0	1	3
inflammation, interstitial	9	3	1	5	3	1	4	4	6	5
dilatation, tubular	0	0	0	2	1	0	0	0	0	2
Lymph node, mesenteric, hemopoiesis	7	5	0	9	9	11	7	12	3	5
angiectasis	1	0	0	0	0	1	0	1	1	2
Pituitary, cyst	0	1	0	1	0	3	2	1	1	1
Rectum, edema	1	1	0	3	0	0	0	0	0	6
erosion/ulceration	0	0	0	0	0	0	0	1	1	1
Spinal cord, cervical, hemorrhage	0	0	0	0	0	0	0	1	0	1
Testis, atrophy, seminiferous epithelium	22	na	21	na	21	na	24	na	27	na
Subcutaneous tissue, hemorrhage	0	0	0	0	0	1	0	1	1	1
Testis, atrophy, seminiferous epithelium	22	na	21	na	21	na	24	na	27	na
Uterus, thrombosis	na	4	na	0	na	4	na	4	na	5

na- not available

Amyloidosis occurred in adrenal, heart, kidney, lacrimal gland, liver, mesenteric lymph node, ovary, parathyroid gland, spleen, thyroid, uterus, vagina and GI tract with higher episodes in the mid- to high dose females. Sponsor considered these incidences are a multisystemic disorder frequently seen in mice, within normal biological variation and no toxicological significance (no historical range provided).

Incidence of Amyloidosis in Various Organs in Albino Mice

Dose, mg/kg	0		0		10		60		400	
	50M	50F	50M	49F	50M	50F	50M	50F	50M	50F
Adrenal	1	1	1	0	2	0	0	1	0	3
Cecum	0	0	1	0	0	0	0	1	0	3
Colon	0	0	1	0	0	0	0	0	0	2
Duodenum	1	0	1	0	1	0	0	1	0	6
Heart	0	0	0	0	0	0	0	2	0	3
Ileum	3	1	1	0	2	0	1	1	0	10
Jejunum	0	0	1	0	0	1	0	1	0	6
Kidney	1	2	1	1	1	5	1	3	0	6
Lacrimal gland	1	0	0	1	0	0	0	0	0	2
Liver	0	1	2	0	2	2	4	0	1	2
Lymph node, mesenteric	0	0	0	0	0	1	0	0	1	1
Ovary	na	1	na	0	na	0	na	3	na	5
Parathyroid	0	0	2	0	1	0	0	2	0	3
Rectum	0	0	0	0	0	0	0	0	0	1
Spleen	0	1	3	0	2	0	3	2	0	2
Stomach	1	0	1	0	0	0	0	2	0	4
Thyroid	0	1	4	0	1	0	1	2	0	4
Uterus	na	1	na	2	na	0	na	3	na	6
Vagina	na	0	na	0	na	0	na	0	na	1

Combined (all organs)	na	**5**	na	**3**	na	**6**	na	**12**	na	**11**

na- not available

Neoplastic findings: None of the individual tumor incidence had either statistically positive trend (p=0.005 for common tumors or p=0.025 for rare tumors) or difference (p=0.01 for common tumors or p=0.05 for rare tumors) compared to each control group (background rate of ≤ 1% for rare tumors). Hemangiosarcomas were found in various organs with increased combined incidence for all treated groups compared to controls, but were not dose-related. The incidence for the high dose group was not statistically significant compared to each control group. Sponsor stated that the tumors were were within the ranges reported in the literature.

Incidence of Primary Hemangiosarcomas in Albino Mice

Dose, mg/kg	**0**		**0**		**10**		**60**		**400**	
	50M	50F	50M	49F	50M	50F	50M	50F	50M	50F
Abdomen	0	1	0	0	0	0	0	1	0	0
Jejunum	0	0	0	0	0	1	0	0	0	0
Liver	0	0	0	1	2	1	0	0	0	3[a]
Lymph node, mesenteric	0	0	0	0	0	0	0	0	1	0
Ovary	na	0	na	0	na	1	na	0	na	0
Spleen	0	0	1	0	1	0	3	1	1	0
Uterus	na	0	na	0	na	2	na	1	na	0
Total	**0**	**1**	**1**	**1**	**3**	**5**	**3**	**3**	**2**	**3**

na- not available

[a]p=0.0734 compared to control 1 with pairwise test

Findings of mass/area raised in the liver at 400 mg/kg correlated with hepatocellular adenomas/ carcinomas more frequently in males, and either individual or combined incidence was not statistically significant compared to each control group.

Non-neoplastic/Neoplastic Findings in the Liver in Albino Mice

Dose, mg/kg	**0**		**0**		**10**		**60**		**400**	
	50M	50F	50M	49F	50M	50F	50M	50F	50M	50F
Area raised	3	na	9	na	6	na	8	na	5	na
Mass	14	na	10	na	17	na	13	na	24	na
Cyst, biliary	0	1	0	0	1	2	1	0	1	1
Hepatocellular adenomas	13	3	15	0	18	2	12	2	25	1
Hepatocellular carcinomas	1	0	4	1	4	0	6	0	5	1
Carcinomas with adenomas	0	0	2	0	1	0	3	0	2	0
Hemangiosarcoma[#]	0	0	0	1	2	1	0	0	0	3
Multiple tumor-bearing animals	2	1	4	1	7	0	4	1	6	0
Adenomas+Carcinomas	14	3	17	1	21	2	15	2	28[a]	2

na- not available

[a]p=0.0209 from control 1 with pairwise test

[#]Rare tumors

Increased alveolar/bronchiolar carcinoma or combined adenoma/carcinoma in the lung was observed from high dose females. The incidence was statistically non-significant with the one-sided trend test compared to each control. However, pairwise comparison revealed a statistical difference at p=0.01 for combined adenoma/carcinoma for the mid- and high dose compared to control group 2 (but not control group 1).

Non-neoplastic/Neoplastic Findings in the Lung in Albino Mice

Dose, mg/kg	0		0		10		60		400	
	50M	50F	50M	49F	50M	50F	50M	50F	50M	50F
Hyperplasia, bronchioalveolar	1	2	2	4	1	2	5	1	4	3
Adenoma, alveolar/bronchiolar	7	12	16	3	10	9[a]	10	11[a]	10	12[a]
Carcinoma, alveolar/bronchiolar	6	1	8	3	2	4	6	7	5	6[b]
Adenomas+Carcinomas	13	13	24	6	12	13	16	18[*]	15	17[*]

[a]p=0.0444 (trend test) or p=0.0913 (LD), p=0.0167 (MD) and p=0.0132 (HD) with pairwise test from control 2
[b]p=0.0433 compared to control 1 with pairwise test
[*]Significantly different from control 2 at p=0.0053 (MD) and p=0.0099 (HD) with pairwise test

Leiomyosarcoma in the uterus was observed with 8% incidence (4/50) in the high dose group with frequent cystic endometrial/stromal hyperplasia. The incidence was not statistically significant compared to each control group. Kidney tubular cell adenoma/carcinoma was associated with frequent incidence of pelvis dilatation and nephropathy. Renal tubular hyaline droplets correlated with systemic neoplasm histiocytic sarcoma. Other rare tumors included malignant meningioma in the brain/optic nerve/pituitary, malignant luteoma in the ovary, islet cell adenoma in the pancreas or squamous cell carcinoma in the stomach observed infrequently (1/50) in the high dose females.

Other Neoplastic Findings in Albino Mice

Dose, mg/kg	0		0		10		60		400	
	50M	50F	50M	50F	50M	50F	50M	50F	50M	50F
Adrenal, benign pheochromocytoma	0	0	0	0	0	0	0	0	0	1
malignant pheochromocytoma	1	0	0	0	1	0	0	0	1	0
Brain, malignant meningioma[#]	0	0	0	0	0	1	0	0	0	1
Hemolymphoreticular tissue,										
histiocytic sarcoma	1	4	0	9	0	10	1	4	0	5
malignant lymphoma	4	6	1	8	6	8	5	10	3	4
Kidney, adenoma, tubular cell	0	0	0	0	1	0	1	0	2	0
carcinoma, tubular cell[#]	1	0	0	0	1	0	0	0	1	0
Optic nerve, malignant meningioma[#], metastasis	0	0	0	0	0	1	0	0	0	1
Ovary, cystadenoma	na	0	na	2	na	2	na	2	na	2
malignant luteoma[#]	na	0	na	0	na	1	na	0	na	1
Pancreas, adenoma, islet cell[#]	0	0	0	0	0	1	0	0	0	1
Pituitary, adenoma	0	2	0	1	0	0	0	3	1	0
malignant meningioma[#], metastasis	0	0	0	0	0	0	0	0	0	1
Spleen, sarcoma[#], metastasis	0	0	0	0	0	1	0	1	0	1
Stomach, carcinoma, squamous cell[#]	0	0	0	0	0	0	0	0	0	1
Testis, adenoma, interstitial cell	0	na	1	na	1	na	1	na	2	na
Uterus, leiomyosarcoma	na	1	na	3	na	0	na	3	na	4
Urinary bladder, mesenchymal tumor[#]	0	1	0	0	0	0	0	1	0	1

[#]Rare tumors
na- not available

SUMMARY AND CONCLUSIONS:

Adequacy of Studies: Sponsor initiated studies based on saturation of absorption of the parent drug at >400 mg/kg/day prior to review by the Executive CAC on the dose selection. The sponsor, however, did not measure either total radioactivity or metabolites for evidence of saturation of absorption. The ongoing studies were considered acceptable by the Exec CAC based on the unbound AUC ratio of >25-fold compared to a 10 mg therapeutic dose. The AUC ratio for the unbound parent drug of approximately 10 fold in both sexes was not sufficient for the high dose of 400 mg/kg/day for mice at the proposed

clinical dose of 20 mg, which is now the sponsor's proposed human dose. Survival rate (~50% overall) was similar in both control and treated groups at the end of the study without marked changes in body weight, and was sufficient for an adequate assessment of tumorigenic potential. Reduced survival rate in the control group 1 during weeks 56 to 100 compared to the other groups did not appear to affect the overall findings. Major cause of death was urinary tract disorders (inflammation/retention) accounting for 35% in males and lymphoreticular neoplasia in 34% of preterminal females. Neither tumor latency nor historical control data from the laboratory were provided in this study.

Non-neoplastic findings: Sponsor considered the increased incidence of the findings was attributed to normal biological variation and no toxicological significance. Gross lesion of soft testes was associated with atrophy of the testicular tubular epithelium and epididymal oligo/aspermia with slight increase from 60 mg/kg. The incidence was often associated histologically in euthanized animals with atrophy of the testicular tubular epithelium. Aspermia in the epididymides was considered as secondary to the atrophy. Macroscopic/microscopic lesions in the cecum/rectum and eye occurred at ≥2 fold in the high dose group compared to controls.

Neoplastic findings: IC351 administration for 2 years caused slight increase in the incidence of hemangiosarcomas in multiple tissues at all doses, lung alveolar/bronchiolar carcinoma or combined adenomas/carcinomas of the mid- and high dose females, hepatocellular adenoma/carcinomas of high dose males, and uterine leiomyosarcoma at high dose. These tumors were not statisticlly significant for the Peto's survival-adjusted one-sided trend test analyzed by Proc Multtest. Marginal statistical difference for the combined adenoma/carcinoma in the lung from control group 2 in the mid-dose ($p=0.0053$) and high-dose ($p=0.0099$) with the pairwise test ($p \leq 0.01$ for common tumors) is not likely to be biologically significant since there was no statistical significance compared to control 1 or combined control group. The hepatocellular tumors were not considered to be biologically significant since both benign and malignant tumors were seen concurrently without significant increase in the number of multiple tumor-bearing animals, and the incidences were not associated with differential cell focus/hyperplasia or preterminal deaths. Other rarely occurring neoplasms included malignant meningioma in the brain/optic nerve/pituitary, islet cell adenoma in the pancreas, squamous cell carcinoma in the stomach and malignant luteoma in the ovary found sporadically (1/50) at high dose females.

Study title: 2-Year Oral Gavage Carcinogenicity Study of IC351 in ——— Wistar Rats

Key study findings: There was a non-significant increase in mammary gland adenocarcinomas in mid dose females, uterine adenocarcinomas at high dose females, and hepatocellular adenomas/carcinomas at high dose males. The AUCs for the unbound parent drug were approximately 14 times in males and 26 times in females the human AUC at the proposed clinical dose of 20 mg.

Study number: — '88203 , —'88779 for —— **Volume #, and page #:** vol. 34
Conducting laboratory and location:
Date of study initiation: November 11, 1997
GLP compliance: yes **QA report:** yes (x) no ( )
Drug: IC351 (LY450190, ———
Lot # (% purity): F96/048A (47.0%), F96/038A (45.5%), M95/118A (47.3%), F96/047A (47.8%), F96/046A (46.5%), 43582 (47.1%)
CAC concurrence: Dose selection was not reviewed by the Executive CAC but the committee concurred on the ongoing carcinogenicity studies on 6/16/99 based on AUC ratios with a 10 mg human dose (see Appendix II for report).

Study Type: 2-year rodent bioassay
Species/strain: ——— Wistar rats (*Rattus norvegicus*) **Number/sex/group:** 50/sex/group
Age at start of study: 6 weeks (133-196 g for males and 100-151 g for females)
Animal housing: Individual

Formulation/vehicle: 0.5% hydroxypropyl methylcellulose (HPMC) containing 1% Tween 80
Drug purity/stability/homogeneity: Accessed
Methods:
Doses: 0, 10, 60 & 400 mg/kg
Basis of dose selection: AUC ratios
Route of administration: Oral gavage
Frequency of drug administration: Daily
Dual controls employed: Dual identical controls
Interim sacrifices:
Satellite PK or special study group(s): 3/sex/timepoint for PK
eviations from original study protocol: N/A
Statistical methods: Proc Multtest implemented with the Peto's survival-adjusted one-sided trend test
Observations and times:

Observations	Times
Mortality/Clinical Signs	Twice daily/Detailed physical exam weekly
Body Weights/Food Intake	Weekly
Food Consumption	Weekly/Monthly after 13 weeks
Ophthalmology	Weeks –1, 52 & 104
Pathology/Clinical Chemistry	Week 104
Toxicokinetics	Days 21, 84 & 180 at 0, 0.5, 1, 2, 4, 8, 16 & 24 hrs post-dose

RESULTS: Mortality rate was 14 to 34% for males and 34 to 46% for females at week 104 (see Appendix III for graphical presentation). Clinical signs of fur staining, skin scabbing/reddening and swollen hindlimbs were noted in addition to decreased activity, labored breathing, dehydration, abnormal gait, hunched posture, weakness, uncoordination and mass (located in the urogenital region) seen in dead animals. There were no treatment-related differences in group mean body weight or food consumption between groups, and occasional statistically significant differences seen early in the study were limited to females, and were not considered to be of biological significance (see Appendix IV for graphical presentation). Plasma exposure with T_{max} values of 2 to 8 hrs, increased less than proportionally to the increase in dose with higher exposure in females. Increase in AUC values was observed on Days 84 and 180, suggesting accumulation of IC351 in the plasma on repeated dosing.

Dose, mg/kg	**0**		**0**		**10**		**60**		**400**	
	50M	50F	50M	50F	50M	50F	50M	50F	50M	50F
Mortality, week 104	7	20	11	17	17	23	16	24	17	21
Clinical Signs,										
Fur staining, dorsal/ventral aspect	17	12	12	16	17	11	20	18	11	24
Fur thin cover, dorsal aspect	4	15	6	21	12	24	10	21	13	33
Skin scabbing, limbs/paws	25	10	21	11	19	7	23	9	19	17
dorsal aspect	9	5	9	6	5	6	11	6	10	11
Abnormal breathing, labored/shallow	3	3	6	4	11	5	7	5	14	1
Abnormal feces, decreased/absent	7	5	5	7	9	11	6	9	3	8
Vaginal discharge	na	2	na	3	na	2	na	3	na	7
Body Weights	UR	UR	UR	UR	UR	UR	UR	UR	UR	UR
Food Consumption	UR	UR	UR	UR	UR	UR	UR	UR	UR	UR
Hematology (%), week 105/106										
Neutrophil segment	37.9	43.2	39.7	42.2	42.6	38.5	39.6	38.8	32.2	38.5
Lymphocytes	58.4	52.9	54.4	54.9	53.8	59.1	57.3	58.4	64.6	58.9
Monocytes	2.0	0.9	2.1	1.1	1.4	1.3	1.6	0.8	1.8	0.8
Eosinophils	1.5	3.0	1.3	1.8	2.2	1.1	1.5	2.0	1.3	1.7
Ophthalmoscopy	UR	UR	UR	UR	UR	UR	UR	UR	UR	UR
Gross pathology	See below		See below		See below		See below		See below	
Toxicokinetics, 3/sex/timepoint										
$AUC_{0\text{-}24hr}$ (ng•hr/mL) Day 21					15040	25012	32822	66695	62821	121462
Day 84					18498	37667	47246	101442	101843	188675
Day 180					16070	35899	38604	91106	78863	152863
C_{max} (ng/mL) Day 21					1398	1995	2626	3834	4856	6678
Day 84					1604	2612	3249	6458	6821	10079

	Day 180			1199	2709	2315	6600	4543	8225
T_{max} (hr)	Day 21			2.0	8.0	8.0	2.0	2.0	8.0
	Day 84			2.0	8.0	8.0	8.0	8.0	4.0
	Day 180			2.0	4.0	8.0	8.0	4.0	8.0

UR- unremarkable
na- not available
Human AUC_{0-24} at steady state= 7,700 ng•hr/mL at 20 mg/day

Non-neoplastic findings: Sponsor considered all the incidences observed in the present study to be spontaneous/incidental or age-related (See Appendix V for incidence of histopathological findings). Dose-dependent multifocal acute hemorrhages in the stomach were observed in the superficial portion of the mucosa of the glandular portion at all groups. Congestion/edema in the lungs increased dose-dependently in females. Higher incidence of thymus cyst in females was associated with thymic atrophy/involution. Dose-dependent increase in hypertrophy and vacuolation of follicular cells in the thyroid was characterized by enlarged epithelial cells with an abundant vacuolated cytoplasm in all female groups. Urinary bladder dilatation/inflammation was observed with increased incidence in the mid- to high dose group males. Increased hematopoiesis in the bone marrow and extramedullary hematopoiesis in the spleen was secondary to the inflammatory or hemorrhagic findings in various organs. Chronic progressive nephropathy was characterized by thickening of glomerular/tubular basement membrane, tubular epithelial basophilia, dilated tubules, interstitial fibrosis and mononuclear cell infiltration. Compression and ventricular dilatation in the brain noted in all groups were secondary to the tumors of the brain or pituitary. Adrenal hypertrophy and pituitary cyst were observed at all doses with increased frequency. Gross lesions of ulceration/scab were correlated with dermatitis. Table below summarizes the microscopic findings with increased incidence.

Non-neoplastic Findings in — Wistar Rats

Dose, mg/kg	**0**		**0**		**10**		**60**		**400**	
	50M	50F	50M	50F	50M	50F	50M	50F	50M	50F
Adrenal, accessory cortical tissue	0	0	0	0	0	0	0	1	0	1
hyperplasia, cortical focal	10	9	1	9	2	8	6	8	7	9
hypertrophy, cortical focal	3	4	3	5	7	9	7	10	8	8
hyperplasia, medullary	0	0	3	2	4	1	3	1	2	1
Eye, degeneration, lens	3	5	3	3	5	4	6	4	6	7
hemorrhage	0	0	0	0	0	0	0	1	0	1
Lung, congestion/edema	1	1	3	3	2	4	2	5	2	7
inflammation, bronchioalveolar	1	0	0	0	0	0	2	0	2	1
Liver, vacuolation, hepatocellular	25	6	17	11	17	8	12	13	20	13
hepatocytic karyomegaly	0	0	0	0	0	0	0	1	0	1
Lymph node, mandibular, congestion	0	0	0	0	0	1	1	2	1	0
mesenteric, dilatation, sinusal	0	0	0	0	1	0	0	0	2	0
pigment deposits	0	0	0	0	0	0	0	1	0	1
hyperplasia, lymphoid	0	0	1	0	0	2	0	0	2	0
Nerve, optic, atrophy	0	0	2	0	0	2	1	0	1	3
Ovary, cyst	na	4	na	8	na	6	na	11	na	9
degeneration, corpora lutea	na	0	na	0	na	0	na	1	na	1
Pituitary, cyst	9	3	9	4	12	3	15	3	19	8
proliferation, tubular	0	1	0	0	0	0	1	0	2	0
Rectum, hyperplasia, mucosal	0	0	0	0	0	0	2	1	1	0
hemorrhage	0	1	1	0	0	0	1	1	1	0
Seminal vesicle, hyperplasia	0	na	1	na	0	na	0	na	2	na
inflammation	0	na	5	na	3	na	4	na	4	na
Skin, dermatitis	0	0	0	0	0	0	0	0	1	1
folliculitis	0	0	0	0	1	1	0	1	0	1
Spleen, cyst, capsular	0	0	0	0	0	0	0	0	1	1

hematopoiesis, extramedullary	5	10	9	8	10	7	12	7	16	11
hyperplasia, lymphoid	0	0	0	2	1	0	1	0	1	0
Stomach, hemorrhage	4	2	6	3	8	9	8	6	10	6
hyperplasia, epithelial	0	0	0	0	1	0	1	0	1	0
ulceration, glandular mucosa	0	1	0	0	0	1	3	1	1	0
Testis, edema	3	na	0	na	1	na	2	na	2	na
polyarteritis nodosa	0	na	0	na	2	na	1	na	2	na
hemorrhage	0	na	0	na	0	na	1	na	1	na
Thymus, cyst	0	9	4	13	2	18	3	21	0	18
Thyroid, dilatation, follicle	0	0	0	1	1	0	1	0	1	0
hemorrhage	0	0	0	0	1	0	1	0	1	0
hyperplasia, follicular cell	3	1	4	0	3	1	4	1	2	3
hypertrophy/vacuolation, follicular cell	3	1	1	2	1	3	7	4	1	6
Urinary bladder, dilatation	0	0	0	1	0	0	5	0	4	0
inflammation	3	2	2	0	6	1	2	2	6	0

UR- unremarkable
na- not available

Neoplastic findings: There was no statistical significance in the incidence of any tumors compared to each control group with either trend or pairwise test.

Increased incidence of hepatocellular adenomas or combined adenomas/carcinomas was sobserved in the high dose male group.

Neoplastic Findings in Liver in — Wistar Rats

Dose, mg/kg	**0**		**0**		**10**		**60**		**400**	
	50M	50F	50M	50F	50M	50F	50M	50F	50M	50F
Adenoma, hepatocellular	2	0	4	1	3	5	3	4	6	2
Carcinoma[#], hepatocellular	0	0	0	0	0	0	1	1	1	0
Adenomas+Carcinomas	2	0	4	1	3	5	4	5	7[a]	2

[#]Rare tumors
[a]p=0.1015 compared to control 1 with pairwise test

Mammary gland adenocarcinoma was associated with mass in the subcutaneous tissue. The tumors were observed in all treated female groups including controls with significantly higher incidence in the mid-dose group, but not dose-related. Pairwise comparison showed statistical significance at p=0.01. Sponsor interpreted increased incidence of mammary gland adenocarcinoma in females to be a chance variation and of no biological significance since the incidence ranged within 0-22% reported in the literature.

Neoplastic Findings in the Mammary Gland in — Wistar Rats

Dose, mg/kg	**0**		**0**		**10**		**60**		**400**	
	50M	50F	50M	50F	50M	50F	50M	50F	50M	50F
Adenocarcinoma	0	1	0	3	0	2	0	10*	0	3

*Statistically significant at p=0.0012 from control 2 with pairwise test

Incidence of uterine adenocarcinomas (10%) was not statistically significant, and considered to be of no biological significance compared to the range of industrial in-house facilities (0 to 20%) for Wistar rats.

Neoplastic Findings in the Uterus in Female — Wistar Rats

Dose, mg/kg, n=50	**0**	**0**	**10**	**60**	**400**
Adenocarcinoma	3	0	1	1	5[a]
Squamous cell carcinoma[#]	0	0	0	0	1
Stromal, polyp	2	4	5	7	6

[#]Rare tumors

*p=0.0208 compared to control 2 with pairwise test

Other rare tumors occurred with IC351 included sarcomas in the colon/pancreas/seminal vesicle, carcinoma in the duodenum, leiomyosarcoma in the jejunum, fibroma/malignant schwannoma in the subcutaneous tissue, and squamous cell papilloma in the stomach observed infrequently (1/50) in the high dose group.

Incidence of Other Neoplastic Lesions in ——— Wistar Rats

Dose, mg/kg	0		0		10		60		400	
	50M	50F	50M	50F	50M	50F	50M	50F	50M	50F
Adrenal, cortical adenoma	0	1	1	2	0	1	0	0	2	1
benign pheochromocytoma	2	0	1	1	0	0	1	1	2	0
malignant pheochromocytoma	0	0	1	0	1	0	2	0	1	0
Colon, sarcoma#, metastasis	0	0	0	0	0	0	0	0	1	0
Duodenum, carcinoma# metastasis	0	0	0	0	0	0	0	0	0	1
Jejunum, leiomyosarcoma#	0	0	1	0	0	0	0	0	0	1
Hemolymphoreticular tissue, malignant lymphoma	0	0	4	0	0	1	4	0	3	1
Lung, carcinoma#, metastasis	0	1	0	0	0	1	1	0	1	1
Lymph node, mesenteric hemangioma	4	0	0	0	1	0	0	0	0	1
Pancreas, adenoma, islet cell	2	0	5	0	7	0	1	2	3	1
sarcoma#	0	0	0	0	0	0	0	0	1	0
Seminal vesicle, sarcoma#, metastasis	0	na	0	na	0	na	0	na	1	na
Skin, adenoma, basal cell#	0	0	0	0	0	0	2	0	0	1
carcinoma, squamous cell	0	0	2	0	3	0	3	0	0	1
Stomach, papilloma, squamous cell#	0	0	0	0	0	0	0	0	1	0
Subcutaneous tissue, fibroma#	0	0	1	0	1	0	0	0	1	0
malignant schwannoma#	0	0	1	1	0	0	0	0	1	0
Thymus, benign thymoma	4	1	1	1	0	3	1	0	2	4
Thyroid, adenoma, C-cell	7	3	2	6	1	0	6	6	3	3
adenoma, follicular cell	4	3	1	0	1	0	4	1	5	1
carcinoma, C-cell	0	2	0	1	0	0	0	0	1	0
carcinoma, follicular cell	0	1	0	1	1	1	0	0	1	0

#Rare tumors
na- not available

SUMMARY AND CONCLUSIONS:

Adequacy of studies: Sponsor initiated studies based on saturation of absorption of the parent drug at >400 mg/kg/day prior to review by the Executive CAC on the dose selection. Sponsor, however, did not measure either total radioactivity or metabolites for evidence of saturation of absorption. Later ongoing studies were considered acceptible by the Exec CAC based on the unbound AUC ratio of >25-fold compared to a 10 mg therapeutic dose. The AUC ratios for the unbound parent drug were approximately 14 fold in males and 26 fold in females the human exposure at the proposed clinical dose of 20 mg. The high dose produced acceptable drug exposure in female rats. Increased mortality in all treated groups from 84- and 48 weeks in males and females, respectively, without significant body weight change, did not appear to affect the overall findings. Neither tumor latency nor historical control data from the laboratory were provided in this study.

Non-neoplastic findings: Sponsor considered all incidences spontaneous, incidental or age-related. However, some incidences occurred at ≥2-fold in the high doses compared to the control groups. These included cortical focal hypertrophy in the adrenal, cyst in the pituitary/thymus, extramedullary hematopoiesis in the spleen, lens degeneration in the eye, stomach hemorrhage, follicular cell hypertrophy/vacuolation in the thyroid, and dilatation/inflammation in the urinary bladder.

Neoplastic finding: The increased incidence of mammary gland adenocarcinoma at mid-dose females, uterine adenocarcinomas at high-dose females, and hepatocellular adenoma or combined adenomas/carcinomas at high-dose males was statistically non-significant and/or within the historical range from the literature. Other rarely occurring tumors included sarcomas in the colon/pancreas/seminal vesicle, carcinoma in the duodenum, subcutaneous fibroma/malignant schwannoma, and squamous cell papilloma in the stomach observed infrequently in the high dose group.

OVERALL INTERPRETATION AND EVALUATION:

Adequacy of the Carcinogenicity Studies and Appropriateness of the Test Model: There was no statistically significant positive trend in survival and statistically significant difference in survival distributions among treatment groups in both mice and rats. The studies were initially conducted based on saturation of absorption at doses >400 mg/kg/day in rats and mice prior to review by the Executive CAC. Later the Exec CAC concurred based on AUC multiples of free drug for rats and mice. Subsequently, the sponsor increased the clinical dose from 10 mg to 20 mg. This resulted in an AUC in humans of approximately 7700 ng•hr/ml (LVDK), nearly 4 times higher than that had been reported for the 10 mg dose. Present studies are not adequate based on PK endpoint since the AUCs for the unbound parent drug below 25 fold in mice (~10 times) for both sexes and male rats (~14 times). It is possible that there is saturation of absorption at doses of ≥400 mg/kg, howerver; parent drug exposure increased slightly with doses up to 2000 mg/kg/day in rats and up to 800-1200 mg/kg/day in mice, and more importantly, there were no data showing that drug metabolites did not accumulate with higher doses.

Evaluation of Non-neoplastic Findings: Sponsor considered the non-neoplastic findings unrelated to treatment with IC351 in both species. Increased incidence of penis protrusion in the high dose mice was consistent with inflammation in urinary tract/prostate/seminal vesicle and/or urinary retention, accounting for 35% of deaths in males. The incidence was often associated with atrophy of the testicular tubular epithelium in euthanized animals. Gross lesion of soft testes was associated with atrophy of the testicular tubular epithelium and epididymal oligo/aspermia with increasing incidence at ≥60 mg/kg. The findings at a NOAEL, which were also present in the dog, were equivalent to 2-fold the human unbound AUC exposures. Macroscopic/microscopic findings in the eye increased in high dose mice and rats compared to the controls, and were also found in the 3-month studies.

Evaluation of Major Tumor Findings: The increased incidences of hemangiosarcoma, mammary gland adenocarcinoma, hepatocellular adenoma/carcinoma, alveolar/bronchiolar adenomas/carcinomas, and uterine adenocarcinoma were either not statistically significant and/or within the ranges reported in the literature.

Biological Significance: The numerical increase (2-fold) in hepatocellular adenoma of the high dose males was was not statistically significant, but seemed to be a treatment-related effect. The histopathological findings of hepatic vacuolation and/or necrosis were also found at ≥60 mg/kg/day in the 3-month mice, at 800 mg/kg in the 3-month rat and at ≥45 mg/kg/day in the 1-month dog studies. The NOAEL for liver tumors (60 mg/kg) produced unbound drug exposure levels 5 fold and 6-14 fold greater in mice and rats than in humans taking 20 mg, respectively.

Potential clinical implications of findings: Clinical implications of the liver tumors in the present studies are not conclusive although there was no evidence of genotoxic or statistical carcinogenic potential. IC351 potentiates the effects of NO by increasing intracellular cGMP levels. NO is an important regulator of hepatocyte function (Biochemistry **63:** 766, 1998). Particularly, generation of NO during inflammation is involved in hepatocellular carcinoma (Mutat. Res. **305:** 253, 1994), suggesting NO as a mediator for cancer development during chronic inflammation. If IC351 does have the potential to cause liver cancer, the mechanism involved is not clear. One possibility is the involvement of

NO/cGMP during inflammation. NO levels are elevated in patients with chronic hepatitis/hepatic tumors (Mutat. Res. **305:** 253, 1994), and the iNOS overexpression during hepatic injury and/or cell necrosis may contribute to the carcinogenesis in the liver (Am. J. Physiol. Gastrointest. Liver Physiol. **281:** G626, 2001). Urinary excretion of cGMP was significantly higher in patients with primary hepatoma and preneoplastic liver disease, suggesting the uptake of cGMP by the liver (Acta Med. Okayama **36:**331, 1982). Selenium may modulate the differentiation and proliferation of tumor cells by reducing cGMP levels (Biol. Trace Elem. Res. **15:** 243, 1988). Adverse events of dyspepsia, and increased frequency of cholecystitis, pancreatitis or GI carcinoma (3 of 1173) in IC351-treated patients (ongoing H6D-MC-LVBL study) may represent inflammatory/precancerous conditions in the digestive tract. GI dilation/ GI tract abnormalities were one of the major drug-related effects for the preclinical/clinical studies of sildenafil. There were elevated AST/ALT levels in some patients dosed with IC351 (H6D-EW-LVAZ, H6D-MC-LVBF, H6D-MC-LVCQ). Newly characterized PDE11A is also present abundantly in liver (J. Biol. Chem. **275:** 314, 2000), and investigation of physiological roles of PDE11A may reveal the function of the subtype regulating cGMP in the liver.

Recommendations for further analysis: The 2-year carcinogenicity studies in male rats, and male and female mice were conducted at doses below those recommended by the ICH guidelines (see Exectutive CAC minutes in appendex II) based on the AUC exposures for the 20 mg human dose. The Committee recommended an additional alternative mouse carcinogenicity assay be conducted for Phase IV commitment unless the sponsor provided evidence for saturation of absorption by measuring either total radioactivity or metabolites.

Labeling Recommendations:

VII. REPRODUCTIVE TOXICOLOGY (see Review #3 for IND 54,553)

Embro/Fetal & Postnatal Development including Maternal Function in the Rat (Segment II & III)

Key study findings: A NOAEL of 200 mg/kg/day was determined for F0 maternal toxicity based on reduced body weight gain, and of 1000 mg/kg/day for F0/F1 reproductive toxicity & for F2 developmental toxicity in rats. A NOAEL for F1 development could not be established due to marked decrease in postnatal survival at all doses from the 1st study, but appeared to be 30 mg/kg in a subsequent study.

Study no.: 353010 & 353016 **Volume #, and page #:** vols. 37/38
Conducting laboratory and location:
Date of study initiation: September 20, 1999 (353010)/April 20, 2000 (353016)
GLP compliance: yes **QA reports:** yes (x) no ( )
Drug/lot #: IC351(LY450190)/#980230 **% purity:** 99.69%
Formulation/vehicle: White powder/10% aqueous acacia

Methods:

Species/strain: Crl:CD®(SD) rats
Doses employed: 0, 60, 200 & 1000 mg/kg/day (353010); 0, 3, 10, 30 & 200 mg/kg/day (353016)
Route of administration: Oral gavage
Study design: F0 females were treated from gestation Day 6 through lactation Day 20.
Number/sex/group: 25/female/group
Parameters and endpoints evaluated: f1 pup developmental evaluations (353010 only), F0 females necropsied on lactation Day 21 or post-mating Day 25, gross necropsies on died or euthanized F1, f2 pups euthanized on PND 7 (353010 only), F1 males for spermatogenesis, F1 females necropsied without mating at the end of mating period (353010 only) & surviving F1 females necropsied on postpartum Day 14 or post-mating Day 25 & F1 males

necropsied —— 353010 only), selected F0/F1/f2 animals for histopathology, TK on gestation Day 19 at 0, 1, 2, 4, 8, 16 & 24 hrs post dosing —— -353016)

Results:

—— 353010:

Body weight gain and food consumption were reduced during gestation Days 6-9/6-20/18-20 in all treated groups, suggesting a test article-related effect. Decrease in food intake during the lactation days 10-14/1-14 was also statistically significant in the mid- to high dose groups.

F0 maternal generation

Dose, mg/kg	**0**	**60**	**200**	**1000**
Mortality, n=25	None	None	None	None
Clinical signs	UR	UR	UR	UR
Body weight change (g), gestation, Days 6-9	14	14	13 (↓7%)	10 (↓28%)
Days 6-20	120	118	115 (↓4%)	109 (↓9%)
Days 18-20	35	30 (↓14%)	27* (↓23%)	28 (↓20%)
Days 0-20	152	147	144 (↓5%)	135 (↓11%)
Food consumption (g/kg/d), gestation, Days 6-9	71	67	64** (↓10%)	61** (↓14%)
Days 6-20	68	67	65* (↓4%)	64** (↓6%)
Days 18-20	66	64	60 (↓9%)	60 (↓9%)
Days 0-20	68	67	65 (↓4%)	64** (↓6%)
lactation, Days 10-14	63	61	54** (↓14%)	54** (↓14%)
Days 1-14	150	147	134** (↓11%)	139 (↓7%)
Pregnancy rate, %	96	96	100	92
total litter loss, lactation Day 1	0	0	0	1
non-gravid	1	1	0	2
Gestation length & parturition	UR	UR	UR	UR
Macroscopic findings	UR	UR	UR	UR
Implantation sites, lactation Day 21	UR	UR	UR	UR

UR-unremarkable

Significantly different from control at p=0.05* or p=0.01**

Pups found dead/missing during the f1 postnatal period (PND 0-21) increased markedly in all treated groups without significant general physical signs and necropsy findings except the presence of milk in the stomach in the treated groups. Postnatal pup survival was significantly reduced during PND 1-4 and birth to PND in all dose groups and below the historical control data range (97.6 & 91.3%, respectively) provided by the sponsor.

f1 litter generation

Dose, mg/kg	**0**	**60**	**200**	**1000**
Mortality (PND 0-21), pups (litters), found dead	5(5)	35(14)	50(15)	40(14)
missing; cannibalized	1	19	36	41
euthanized in extremis	0	0	0	1
Clinical signs	UR	UR	UR	UR
Body weights	UR	UR	UR	UR
PND 0 Litter, pups born/litter, live litter size, % males at birth	UR	UR	UR	UR
Postnatal survival, %/litter, PND 1-PND 4	99.1	91.5**	84.5**	85.8**
birth-PND 4	98.4	87.1**	81.9**	79.5**
Macroscopic findings, Stomach, milk present, found dead litters (pups)	0/5 (0/5)	5/14 (5/35)	2/15 (3/50)	4/14 (4/40)

UR- unremarkable

Some animals were found dead or euthanized *in extremis* in the mid- and high dose groups. Decreased body weights observed in PND 21-28, PND 49-56 and gestation Days 15-18 were not considered to be

test article-related. Sperm evaluation data was within the historical values provided by the sponsor. Microscopic findings were considered to be spontaneous, incidental or normal background changes.

F1 generation postnatal deveolpment

Dose, mg/kg	**0**		**60**		**200**		**1000**	
	24M	24F	24M	24F	25M	25F	22M	21F
Mortality, found dead	0	1[A]	0	0	1[B]	0	0	1[D]
euthanized in extremis	0	0	0	0	1[C]	0	0	2[E]
Clinical signs	UR	UR	UR	UR	UR	UR	UR	UR
Body weights, PND 21-28	40.1	33.4	36.8	32.6	35.8*	30.9	35.7*	30.6
PND 49-56	62.6	29.2	61.2	26.9	62.9	30.1	59.0	23.7*
gestation, Days 15-18	na	40	na	42	na	36	na	29*
Developmental sensory function & behavior[#]	UR	UR	UR	UR	UR	UR	UR	UR
Reproductive performance[@]**, fertility index (%)**	91.3	95.7	91.7	95.8	87.0	88.0	90.5	90.5
Gestation length & parturition	na	UR	na	UR	na	UR	na	UR
Spermatogenic endpoint evaluations[$],								
Sperm counts, left testis, no. sperm/10^6/g	95.5	na	87.6	na	91.5	na	84.5	na
left epididymis, no. sperm/10^6/g	476.1	na	421.0	na	428.4	na	433.7	na
Sperm production rate, no. sperm/10^6/g	15.7	na	14.4	na	15.0	na	13.8	na
Sperm morphology, head absent/normal flagellum, %	0.0	na	0.1	na	0.0	na	0.4	na
Implantation sites, lactation Day 14	na	UR	na	UR	na	UR	na	UR
Organ weights, females- fail to deliver, uterus/CX/OD (g)	na	na	na	0.90 (n=1)	na	1.11 (n=2)	na	0.69 (n=2)
Macroscopic findings,								
Liver, white area, lactation Day 14	na	0/21	na	0/23	na	0/22	na	1/17
Urinary bladder/ureter[W], scheduled necropsy	0/24	na	0/24	na	0/23	na	1/22	na
Microscopic findings,								
Kidneys, inflammation, chronic active, moderate	0/2	na	na	na	0/1	na	1/2	na
hyperplasia, transitional cell, mild	0/2	na	na	na	0/1	na	1/2	na
Prostate, inflammation, chronic active, moderate	0/24	na	na	na	na	na	1/22	na

UR-unremarkable
na- not available
Died on Week 19[A], during Week 12[B] or during Week 21[D] (postpartem Day 10)
[C]Euthanized *extremis* during Week 14 due to impaired mobility, labored respiration & swayed while walked.
[E]Euthanized *extremis* in one female during Week 8 (PND 35) due to dehydrated/lethargic, labored respiration & moderate to severe matting on various areas on the body or in the other during Week 9 (gestation Day 22) due to unkempt appearance, decreased defecation, dried red staining on the mouth/nose/forelimbs, & dehydrated, which was diagnosed to be dystocic.
[#]Pinnal detachment, surface righting response, eye opening, balanopreputial separation, vaginal patency, auditory startle test, motor activity & Biel maze swimming trials for learning/memory ability were tested.
[@]Fertility, mating index & estrous cycle were tested.
[$]Testicular/epididymal sperm numbers, sperm repoduction rate, sperm mobility & % of morphologically normal sperm were tested.
[W]Urinary bladder distended/calculi/thickened/reddened mucosa/red fluid contents or ureter distended
Significantly different from control at p=0.05* or p=0.01**

f2 litter generation; There were no remarkable effects of IC351 on the mortality, clinical signs, body weights, PND 0 litter data, postnatal survival & macroscopic findings at necropsy (PND 7) in f2 generation.

— **353016:**

F0 maternal generation; Oral administration of IC351 had no adverse effects on F0 survival, body weights/food consumption, gestation, parturition/maternal function during lactation & macroscopic findings at 3, 10, 30 & 200 mg/kg/day. Plasma exposure was less proportional to the increase in dose, which was consistent with previous studies in the pregnant and non-pregnant rats. Higher exposure was observed in the 200 mg/kg/day group on gestation Day 19 in the present study than on gestation Day 12

in the previous study ($AUC_{1\text{-}24h}$= 63554.5 ng•hr/mL, # —-353005). Sponsor considered that the different gestation day may affect the exposure to IC351.

Dose, mg/kg, n=23-25	**0**	**3**	**10**	**30**	**200**
Body weight changes, lactation, Days 1-21	32	30	34	38	47*
Toxicokinetics, $AUC_{0\text{-}24h}$ (ng•hr/mL)		10614	31686	55590	91115
C_{max} (ng/mL)		912	1872	3490	6584
T_{max} (hr)		4	4	8	8

*Significantly different from control at p=0.05

f1 generation: Mean postnatal survival rate for the birth to PND 4 in the 30 mg/kg group was below the sponsor's contol data of 91.3% due to the deaths of 7 pups from 1 litter during this interval according to the sponsor.

Dose, mg/kg	**0** 23M 23F	**3** 23M 24F	**10** 24M 24F	**30** 25M 24F	**200** 24M 24F
Mortality, pups (litters), found dead	12(7)	13(8)	13(8)	27(9)	12(8)
euthanized in extremis	0	0	0	4	0
missing; cannibalized	3	3	3	12	6
Clinical signs	UR	UR	UR	UR	UR
Body weights	UR	UR	UR	UR	UR
PND 0 Litter#	UR	UR	UR	UR	UR
Postnatal survival, birth-PND 4 (%/litter)	96.1	96.4	96.6	89.8	96.5
Macroscopic findings, litters					
Lungs, reddened	0/22	1/24	1/24	3/23	2/24

UR-unremarkable

#Pups born/litter, live litter size & % males at birth were studied.

Summary: — **-353010;** F1 generation was likely to be exposed *in utero* since parent and/or metabolites of IC351 were detected in maternal placenta and fetal tissues (Study #003R00). No mortality was observed in F0 maternal & f2 generations, but pups or litters found dead increased markedly at all doses during f1 postnatal period (PND 0-21) with one pup in the high dose euthanized *in extremis* without general clinical signs except the presence of milk in the stomach in some of the treated groups. Some rats in F1 generation in the mid- and high dose groups were found dead or euthanized *in extremis*. Sponsor considered the effects not treatment-related since no deaths occurred in the high dose males. Dose-dependent reduction in body weight gain and food consumption for F0 were observed during gestation Days 6-9, 6-20 & 18-20 in all treated groups, suggesting a test article-related effect. Decreased food consumption during lactation days 10-14/1-14 was statistically significant in the mid- to high dose groups, which was attributed to a reduced maternal nutritional demand caused by an increase in pup deaths during PND 1-4 as explained. Reduced F1 mean body weight also occurred sporadically in the mid- to high dose group, which was considered to be unrelated to treatment. Drug exposure via milk is likely to be minimal since maternal milk is not a major route of elimination (≤0.1% in the rat) for IC351 and/or its metabolites (Study #002R00). One male in the high dose group exhibited a distended ureter, dilated renal pelvis, a distended/thickened urinary bladder with calculi/reddened mucosa, chronic inflammation/hyperplasia in the kidney at a scheduled necropsy, and considered to be spontaneous. Postnatal pup survival was significantly reduced during PND 1-4 and birth to PND 4 in all dose groups, and below the sponsor's historical control data. There were no TK data for the study. — **353016;** Another postnatal growth/survival and TK study at dose levels of 3, 10, 30 & 200 mg/kg/day were conducted to determine the reproducibility and a NOEL. The reduced f1 postnatal survival was not replicated except in the 30 mg/kg/day with a survival rate of 89.8% slightly below the sponsor's historical control data of 91.3%. Sponsor considered a NOEL for F1 developmental toxicity as 30 mg/kg/day.

Conclusion: Administration of IC351 to F0 dams did not affect F1 male/female reproductive performance and fertility. The NOAEL for F0 maternal toxicity was selected at 200 mg/kg/day based on the reduced body weight change/food consumption during gestation. A NOAEL for F0/F1 reproductive toxicity & for F2 developmental toxicity was identified as 1000 mg/kg/day. A NOAEL for F1 developmental toxicity in the rat could not be established in the combined segment II/III study due to statistically significant decrease in postnatal survival at all dose groups. In a subsequent study (——→ 353016), postnatal survival was not greatly affected at doses of 3, 10, 30 & 200 mg/kg, and the sponsor suggested the NOEL for F1 developmental toxicity was 30 mg/kg/day. The discrepancies of the findings were not clear since both studies were conducted the same way except for dose selection.

Reproductive toxicology summary: Developmental and reproductivity studies were conducted in rats & mice (refer to Reviews #2 & 3 ———————— Mice were selected as a second species for the embryotoxicity studies due to the poor plasma exposure in rabbits (Study #B00199). Although the drug was not directly administered to the F1 neonates, it is likely they were exposed since IC351 crosses the placenta (Study #003R00). Administration of IC351 had no adverse effects on fertility or reproductive toxicity at doses up to 400 mg/kg/day in either male or female rats. A NOAEL for maternal toxicity (based on the reduced body weight gain) was determined to be 200 mg/kg/day and for embryo/fetal developmental toxicity to be 1000 mg/kg/day in rats. Fetal skeletal malformations/variations in all groups including controls in both rats and mice were within the provided historical data. No other developmental malfunction was noted. A NOAEL for both maternal and developmental toxicity was established as 1000 mg/kg/day in mice. Present studies of combined perinatal/postnatal development indicated that oral administration of IC351 did not adversely affect F0 gestation or parturition up to 1000 mg/kg/day, but significantly reduced the postnatal survival at all doses studied. The cause of death was not determined. Developmental sensory function/ behavior, reproductive performance, implantation sites, spermatogenic endpoints, and mean organ weights in the F1 pups were not markedly affected by maternal treatment up to 1000 mg/kg/day. The number of f2 pups born per litter and postnatal pup survival were also not affected by F0 maternal treatment. The sponsor suggested a NOEL for f1 developmental toxicity to be 30 mg/kg/day. This represents a 9-fold exposure multiple for unbound parent drug compared to the human exposure at a 20 mg dose.

Reproductive toxicology conclusions: There were no significant differences in fertility/mating indices, sperm counts, motility and morphology in IC351-treated male rats up to 400 mg/kg/day. However, Increased incidence of non-reversible testicular seminiferous atrophy/regression and decreased sperm count/aspermia was observed from 10 mg/kg/day in the 3- and 6-month studies and ≥25 mg/kg in the 1-year study for dogs, and 800 mg/kg in the 3-month and ≥60 mg/kg in the 2-year studies for mice. No adverse effects on the estrous cycle, mating, fertility, ovarian weights, numbers of corpora lutea, implantation sites, resorptions and embryos were observed in female rats up to 400 mg/kg. A NOAEL for maternal toxicity in rats was considered to be 200 mg/kg/day based on the decreased body weight gain from both previous and present studies. In mice, a NOAEL of 1000 mg/kg was established for maternal and developmental toxicity since all findings were within the provided historical control data. F1 male and female reproductive performance and fertility were not significantly affected by F0 maternal treatment up to 1000 mg/kg/day. No adverse effects were observed in F1 gestation/parturition/lactation or in f2 pups born per litter/postnatal pup survival. A NOAEL for F0 & F1 reproductive & F2 developmental toxicity in rats was identified as 1000 mg/kg/day. A NOAEL for F1 developmental toxicity in the rat could not be established in the combined segment II/III study due to the statistically significant decrease in postnatal survival in all dose groups. In a subsequent study, however, the effect was not observed at 3, 10, 30 & 200 mg/kg, suggesting equivocal toxicological significance.

Labeling recommendations: ————————————

————————————

VIII. SPECIAL TOXICOLOGY

The ocular irritation potential of IC351 was assessed in *in vitro* and *in vivo*, and found to be a mild irritant to the ocular tissue of the rabbit *in vivo*. The *in vivo* acute dermal toxicity was evaluated in rabbits, and determined to be a slight irritant at 1000 mg/kg.

IX. DETAILED CONCLUSIONS AND RECOMMENDATIONS:

IC351 was tested in several oral formulations due to the low water solubility and incomplete oral absorption in animals. A ——— of IC351 and was generally used for earlier pharm/tox studies including carcinogenicity studies. A suspension of micronised formulation in 10% acacia for rodents or CMC/SLS for the 1-year dog study, however, did not achieve higher exposure than the ——— Sponsor indicated that dose level was adjusted for adequate exposure depending on the formulation.

IC351 potentiated the relaxant effect of NO in human penile resistance arteries and cavernosal trabecular smooth muscle by inhibiting the hydrolytic inactivation of cGMP by PDE5. It does not increase cGMP levels in cardiac myocytes nor alter the contractile response of papillary muscle in rats. IC351 was 700-fold and its intermediate metabolites were 90- and 250-fold less active against the human photoreceptor PDE6 than against PDE5. IC351 is >10,000-fold more potent for PDE5 than PDE3, an enzyme involved in cardiac contractility. IC351 retains relatively low selectivity for PDE5 vs. human PDE11A (abstract from Am. Coll. Clin. Pharmacol., VA, 2001), which was widely expressed in kidney, liver, pituitary/salivary glands and testis (PNAS 97: 3702, 2000). Thus, pharmacological characterization of IC351 on human PDE11A may provide further clarification of the mechanism of IC351, although the physiological roles of PDE11A are not yet known.

General safety pharmacology studies were conducted in mice, rats, dogs and guinea pigs. Oral administration of IC351 produced a slight-to-moderate ptosis and depression of the pinnal reflex in rats exposed to 200 mg/kg, and vomiting/increased heart beat at ≥10 mg/kg followed by tarchycardia at ≥30 mg/kg in dogs (1/2 at 30 mg/kg and 2/2 at 100 mg/kg). IC351 at oral doses of 1- and 5 mg/kg produced significant, long-lasting reductions in blood pressure in hypertensive rats, and to a lesser extent in normotensive rats. In anesthetized dogs, i.v. administration of IC351 at ≥0.1 mg/kg produced a dose-dependent reduction in blood pressure in the presence of decrease in total peripheral resistance. At oral doses of 20- and 200 mg/kg, decrease in mean arterial blood pressure was observed in conscious dogs without effects on heart rate or respiration rate. The cardiovascular effects, however, were not observed in the repeated dose toxicity studies, partly due to the recording time of 2- to 4 hrs after dosing which is not consistent with maximum plasma concentrations due to high variability (T_{max} ranged from ——— hrs). Significant diuresis and natriuresis were observed in rats at 0.3 mg/kg (i.v.). Repeated daily oral dosing for 21 days produced body weight loss, deteriorated health, bradycardia/heart block, and deaths at 400 mg/kg in conscious guinea pigs. Sponsor considered the ECG changes occurred as a result of the

deteriorated clinical condition of the animals since the effects occurred in the absence of or modification to t and p waves.

Renal clearance is negligible in rats (4 to 6%) and higher in female dogs (25%). Oral bioavailability was 34- to 53% in rats and 10- to 18% in dogs with low plasma clearance, suggesting an incomplete first pass metabolism and the possibility of prolonged absorption. After 24 hrs, the majority of the radioactivity was associated with metabolites in human feces. Exposure increased proportionally over a dose range of 2.5- to 20 mg with a $t_{1/2}$ of 17.5 hours in human. Steady-state plasma concentrations were attained by Day 5 with doses of 10- or 20 mg/day.

The major metabolite in human and dog liver slices is a methylcatechol glucuronide. The same metabolite was present in the rat and mouse in addition to a hydroxyglucuronide and a catechol glucuronide, which was most abundant in the rat. The major glucuronide metabolite in human plasma and urine would be unlikely to have clinically significant effects on any of the human PDEs tested at therapeutic doses based on the known plasma concentrations of the metabolite observed in humans. IC351 was found to be an inducer of CYP1A/CYP2B in mice and rats, and a mechanism-based inactivator of CYP3A in male mice. CYP3A4 was found to be the primary P450 isozyme responsible for the biotransformation of IC351 to the methylcatechol and catechol metabolites.

In rats, the highest concentrations of radioactivity were detected in the GI tract, stomach, adrenals, liver, pancreas, kidneys, lymph nodes, thyroid and lung with greater than plasma concentrations in most tissues after an hour following a single oral dose of 10 mg/kg [^{14}C]-IC351. Twenty four- and 168 hours after dosing, radioactivity was found in the stomach/GI and liver, respectively. The tissue half-life was approximately 10 hours in most tissues except for whole blood (26 hours) and stomach wall (21 hours). There was minimal radioactivity in the CNS in rats. *In vitro* binding of IC351 to human, rat, dog and mouse plasma proteins was determined to be 94%, 92%, 87% and 85%, respectively. Feces was the major route of elimination of radioactivity in humans, rats and dogs, and the majority of radioactivity was excreted in the feces with a recovery of 86% within 72 hours in the rat, indicating incomplete oral absorption and biliary excretion of metabolites.

Daily oral administration of IC351 up to 6 months in mice and rats and up to 1 year in dogs demonstrated that plasma exposure generally increased sub-proportionally to the dose with variable T_{max} of 1 to 24 hours possibly due to variable absorption and saturation of absorption. Plasma AUC values were higher after 1 to 3 months in rats and dogs, suggesting accumulation in the plasma. The AUC and C_{max} values decreased after 1 month in mice, suggesting enzyme induction following multiple dosing. The 6- and 12-month chronic dosing in the dog, however, showed no consistent trends, and there was considerable intra-animal variability in AUC values.

Single dose toxicity was evaluated in mice and rats up to 2,000 mg/kg orally and 100 mg/kg intravenously. IC351 did not cause death at 2,000 mg/kg in mice and rats. The i.v. lethal dose was 100 mg/kg in mice and >62.5 mg/kg in rats. Significant signs of toxicity included labored breathing, jerky movements, subdued behavior, prostration, tremor and convulsions with intravenous doses of ≥37.5 mg/kg in both species. Sponsor considered the majority of clinical signs attributable to the vehicle used (90% PEG).

Maximum tolerated dose toxicity was studied with escalating daily oral doses of spray-dried (0.5% HPMC/1% Tween 80) IC351 up to 2,000 mg/kg/day in rats and up to 800 mg/kg/day in dogs. There were no treatment-related deaths. In rats, clinical signs included reflux of dose, noisy breathing, vocalizing and salivation at 2,000 mg/kg probably due to a large dosing volume. Loose feces (100 mg/kg), vomiting/subdued behavior (200 mg/kg), and pale feces (800 mg/kg) were the major clinical signs observed in dogs with body weight loss at high dose. Thymic atrophy and inflammatory infiltrates in the

stomach/liver were noted. Sponsor considered the maximum repeatable daily doses of 400 mg/kg in rats and 200 mg/kg in dogs with respect to the saturation of plasma absorption.

Repeated dose toxicity was studied for two 3-month studies in mice, 1-, 3-, and 6-month studies in rats, and one 1-, two 6- and one 12-month studies in dogs. In the first 3-month study in CD-1 mice, there was bone marrow hypocellularity, corneal mineralization, liver necrosis, lymphoid atrophy/necrosis in the spleen/thymus at 400 mg/kg given as a ———— with HPMCP. Hepatic vaculation occurred in all treated groups. Daily doses up to 800 mg/kg of IC351 in 10% acacia were administered in the 2nd study. Increased incidence of lymphoid necrosis in the thymus and periarteritis in the testicular arteries was observed at 800 mg/kg. Splenic hematopoiesis was noted in all treated groups. Unilateral hypospermatogenesis and renal tubular inflammation occurred at 800 mg/kg/day.

IC351 was well tolerated in rats at doses up to 400 mg/kg in the 1-, 3- and 6-month studies. Major findings of the 1-month study (— Wistar rats) were thymic periarteritis with hemorrhage foci, perivascular eosinophilic inflammation in the lung, and splenic pigmented macrophage observed in the high dose group given 0.5% HPMC/1% Tween 80 as a vehicle. In the 3-month study (Fisher rats), one male had hepatic necrosis/vacuolation and 1 female had a periarteritis in the mesenteric arteries with multifocal peritonitis at 800 mg/kg given in 10% acacia. Minimal to slight chronic periarteritis characterized by infiltration of the tunica adventitia and media by lymphocytes and monocytes and minimal endothelial hypertrophy and lymphocytic infiltrates in the perivascular connective tissues surrounding the testicular and hepatic arteries were noted with increased incidence at high dose. Histopathologic findings in the 6-month study in — Wistar rats given IC351:HPMCP (———— included brown pigment deposition in hepatocytes/Kupffer cells without splenic hemosiderin deposition at ≥60 mg/kg, and periphlebitis in the mesenteric veins with decreased lymphocytes (males) at all treated groups. Regenerative tubular epithelium/pelvic epithelial hyperplasia in the kidney, hepatic arteritis, hemorrhages in the thymus/lymph nodes and splenic extramedullary hematopoiesis were observed with increased frequency at the high dose.

One high dose male dog (200 mg/kg) in the 1-month study (given in 0.5% HPMC/1% Tween 80) was killed *in extremis* on Day 14 due to ill health secondary to the dissseminated arteritis in the brain, spinal cord, lungs and thymus. This dog showed elevations of neutrophils/total leukocytes/monocytes/ fibrinogen. Other dogs exhibited higher incidence of clinical signs of thin appearance, subdued behavior and loose feces from 45 mg/kg. Statistically significant reduction of body weight gain was observed in the first week at high doses and remained throughout the study. There was an elevation of ST segment indicative of myocardial infarction, pericarditis and myocardial hypoxia in high dose females (2/3). A supplementary examination of the hearts revealed coronary arteritis at ≥45 mg/kg in 1 of each male and female dog. This lesion was not associated with vasodilation/tachycardia but with moderate decrease in heart rate.

A 3-month toxicity study was conducted in male dogs only with IC351:HPMCP ———— at 0, 10, 60 or 200 mg/kg/day in gelatin capsules. Severe bone marrow myelopoisis associated with histiocytic cell infiltration and increased extramedullary hematopoiesis in the liver or spleen were found at the high dose. These dogs also had myocardial degeneration/fibrosis and epicarditis with ulceration/inflammation of the GI tract. Degeneration of the seminiferous epithelium characterized by missing germ cells and/or spermatids was observed in the high dose group during treatment and following recovery. Other histopathologic findings included an accumulation of fine brown pigment in the epithelium of the gallbladder with increased frequency at ≥60 mg/kg.

Two 6-month toxicity studies at oral doses of 0, 10, 60 and 400 mg/kg formulated in IC351:HPMCP ———— were conducted since the first study had arteritis effects considered secondary to Beagle Pain Syndrome (BPS) in the specific colony supplied, and testicular findings due to use of sexually immature

dogs according to the sponsor. High dose dogs (4/6) were killed moribund due to recurrent (≥5) episodes of symptoms of BPS. Clinical signs including thin appearance, subdued behavior, stiff neck and pyrexia developed in 1 mid dose and 6 high dose dogs. Significant body weight loss was noted in the-mid and high dose dogs during the first 12 weeks of the study. Elevated neutrophils/WBC/monocytes/fibrinogen levels and decreased APTT/albumin/calcium levels were seen in the high dose group, consistent with polyarteritis. Increased ALP in the-mid to high doses and decreased RBC/Hb in the high doses were observed and did not return to normal by the end of the recovery period of 1-month. Arteritis was observed in multiple tissues including the spinal cord, thymus, brain, esophagus, urinary bladder, heart (coronary arteries), lungs, ovaries, epididymides, mammary gland and stomach with increased frequency in the high dose group. The pathologist concluded that the effect was either a treatment-related change or exacerbation of spontaneous polyarteritis. Although therapeutic ratios between the exposure for targeted pharmacological activity and the vasculitis could not be determined, a NOAEL of 60 mg/kg produced 3- to 33-fold exposure multiples (due to individual variability) for the unbound parent drug above the human exposure at 20 mg. The second study, however, did not evaluate all target tissues for arteritis. Irreversible testicular atrophy associated with oligo/aspermia was observed with increased frequency at ≥60 mg/kg. The NOAEL gave equivalent exposure for unbound parent drug at a 20 mg clinical dose.

One-year chronic toxicity was conducted in beagle dogs dosed with IC351 in gelatin capsules containing 1% CMC/0.5% SLS at 0, 25, 100 or 400 mg/kg/day. Pale feces were the most frequent clinical signs at all doses. Mean body weight for females in the mid- and high doses was decreased by 6 weeks after the study. Treatment was suspended for 1 of 100- and 1 of 400 mg/kg females between Days 140 and 166, and from Day 196 through the end of the study, due to marked neutropenia with moderate thrombocytopenia. The changes returned to within reference intervals in 2 to 3 weeks after drug removal, but appeared again within 2 weeks after reinitiation of IC351. The 400 mg/kg female had clinical signs of pyrexia, anorexia and lethargy with no vascular lesions but had a single focus of perivasculitis in the circumflex branch of the left coronary artery. Sponsor considered the findings to be idiosyncratic and not a result of a direct toxicity on bone marrow with no inhibition in neutrophil precursors or megakaryocytes. Degeneration and atrophy of the seminiferous epithelium were observed in all treated groups with decreased testicular weight. Aspermia was noted in the epididymides of the most severely affected dogs, and the incidence and severity of the lesions increased following 12 months of dosing. Other histopathologic findings included lymphocytic infiltration in gall bladder and multifocal fibrosis in the prostate observed sporadically at all treated groups. Hepatic leukocytosis/perivasculitis/pigmentation were noted with slightly increased frequency in the high dose group.

Genotoxicity was evaluated in the standard battery of Ames test, a mammalian cell mutation test in mouse lymphoma cells and a cytogenetic test in human peripheral lymphocytes in the presence or absence of metabolic activation as well as World Health Organization (WHO) nitrosation assay *in vitro*. IC351 was also tested in rat bone marrow micronucleus test *in vivo*. IC351 was not cytogenetic, clastogenic or cytotoxic with no evidience of intrinsic genotoxic potential.

Two-year carcinogenicity studies of IC351 given in 0.5% HPMC/1% Tween 80 were initiated by the sponsor in CD-1 mice and — Wistar rats at 0, 10, 60 and 400 mg/kg/day based on saturation of absorption at doses >400 mg/kg/day prior to review by the Executive CAC. The Exec CAC concurred with the doses based on AUC multiples of free drug for rats and mice. Subsequently, the sponsor increased the clinical dose from 10 mg to 20 mg. This resulted in an AUC in humans of approximately 7700 ng.hr/ml (LVDK), nearly 4 times higher than that had been reported for the 10 mg dose. The high dose produced systemic exposure of 10 fold and, 14- and 26 fold in mice and rats, respectively, compared to the human dose of 20 mg, indicating that for mice and male rats adequate exposure was below the 25-fold animal/human ratio recommended by the ICH guidelines. In addition, parent drug exposure increased slightly with doses up to 2000 mg/kg/day in rats and up to 800-1200 mg/kg/day in mice, and

more importantly, there were no data showing that drug metabolites did not accumulate with higher doses.

Major non-neoplastic lesions observed with increased frequency in IC351-treated mice included (1) penis protrusion in the high dose group consistent with inflammation in urinary tract/prostate/seminal vesicle and/or urinary retention, accounting for 35% of male deaths during the study, (2) gross lesion of soft testes associated with atrophy of the testicular tubular epithelium and epididymal oligo/aspermia at ≥60 mg/kg, (3) dark areas/foci in the cecum or edema of the cecum/rectum in the high doses paralleled the amyloidosis in the digestive tract, (4) lens degeneration/corneal mineralization/erosion in the eye at 400 mg/kg and eye opacity at ≥60 mg/kg, and (5) hepatocellular vacuolation correlated with mass/area raised in the liver at at all doses in females.

Major neoplastic findings in mice were hepatocellular adenomas/carcinomas in high dose males, hemangiosarcomas in multiple tissues at all doses and alveolar/bronchiolar adenomas/carcinomas in mid dose females. Mammary gland adenocarcinomas of mid dose females, and hepatocellular adenomas/carcinomas at high dose males were observed with increased frequency in rats. The increased incidence of the tumors was not statistically significant.

Reproductive and developmental toxicity of IC351 given in 10% acacia was assessed in CD-1 mice and SD rats. Mice were used for a second rodent species of embryo/fetal development studies since plasma exposure for rabbits was minimal due to either extensive first pass metabolism or poor absorption. There were no significant differences in fertility/mating indices, sperm parameters (count, motility and morphology) in IC351-treated male rats up to 400 mg/kg/day. However, non-reversible seminiferous tubular atrophy/regression and/or decreased sperm count/aspermia were observed from 10 mg/kg/day in the 3- and 6-month, from 25 mg/kg/day in the 1-year for dogs, and at 800 mg/kg in the 3-month and from 60 mg/kg for the 2-year studies in mice with low multiple exposure (<10-fold) at a 20 mg/day clinical dose. No adverse effects on the estrous cycle, mating, fertility, ovarian weights, numbers of corpora lutea, implantation sites, resorptions and embryos were observed in female rats up to 400 mg/kg. A NOAEL for maternal toxicity in rats was considered to be 200 mg/kg/day based on the decreased body weight gain. A NOAEL of 1000 mg/kg was established for maternal and developmental toxicity in mice. F1 male and female reproductive performance and fertility were not significantly affected by F0 maternal treatment up to 1000 mg/kg/day in rats. No adverse effects were observed in F1 gestation/parturition/ lactation or in f2 pups born per litter/postnatal pup survival. A NOAEL for F0 & F1 reproductive & F2 developmental toxicity in rats was identified at 1000 mg/kg/day. A NOAEL for F1 developmental toxicity in the rat could not be established in the combined segment II/III study due to the statistically significant decrease in postnatal survival in all dose groups. In a subsequent study, however, the same effect was not observed at 3, 10, 10, 30 & 200 mg/kg, suggesting an equivocal toxicological significance. Sponsor considered a NOAEL for a developmental toxicity to be 30 mg/kg/day.

Plasma exposure of IC351 was measured in pregnant mice and rats during gestation and was less than proportional to doses over the range of 60 to 1000 mg/kg, a result also seen in non-pregnant animals. Milk excretion and placental transfer were determined with 10 mg/kg of [^{14}C]-IC351 in pregnant Fischer rats. The radioactivity in maternal milk was less than 0.1%, suggesting that maternal milk is not a major route of elimination and the exposure through maternal milk would be insignificant. IC351 crosses the placenta resulting in fetal tissue exposure, which is substantially lower than maternal exposure.

Special toxicity studies of *in vitro* and *in vivo* ocular irritation or *in vivo* dermal irritation were conducted. IC351 is considered as a mild ocular and dermal irritant in New Zealand White rabbits *in vivo*.

Conclusions: The preclinical toxicology studies conducted with IC351 demonstrate that the drug is generally well-tolerated in mice, rats and dogs.

General Toxicology Issues: Major histopathological findings of IC351 treatment in animals are arteritis and testicular degeneration/atrophy, which were also found in other PDE 5 inhibitors. The testicular findings were observed with increased incidence in mice in the 3-month toxicity study and the carcinogenicity study, and in dogs in the 3-, 6- and 12-month toxicity studies with no/low safety margin compared to the proposed clinical dose of 20 mg. The findings are likely to be irreversible since the incidence was observed during the recovery in the 3- and 6-month dog studies. Sponsor considered that reversibility is unlikely although the reversibility of the finding is unknown for the 1-year study, extensive cell loss in the germinal epithelium was observed in dogs with the most severe testicular alterations. In men, there were no clinically significant effects on semen parameters up to 6 months at 20 mg (#H6D-MC-LVCZ).

Vasodilators such as phosphodiesterase type-III inhibitors are known to cause vasculitis in rats and dogs. In dogs, vasodilators produce a cardiovascular toxicity, which generally includes coronary arteritis and more variably, atrial epicardial hemorrhage and/or focal myocardial necrosis. The arteritis tends to resolve by fibrosis and intimal hypertrophy. A second class of lesions features non-hemorrhagic inflammation, with or without fibrinoid necrosis, of one to three layers of the wall of arterioles and venules, and typically manifested as rash or purpura, although viscera can be involved. The lesions are believed to reflect hypersensitivity, and are the major manifestation of clinical drug-induced vasculitis (Drug-induced vasculitis: interim guidance for industry and FDA pharmacologists Jan. 2002). The relevance to humans and the pathogenic mechanism of drug-related vascular lesions in animals are poorly understood, and the specific biomarkers are not identified. Vasculitis was observed in IC351-treated dogs, mice and rats. For the most part, vascular effects were slight to minimal, not particularly dose-related, and occurred at high doses with exposures to free drug much higher than exposures in men. In rats and mice, IC351 caused minimal to slight vasculitis with moderate multiples of human exposures. In only one instance were there any effects (phlebitis of mesenteric arteries) at doses below the highest dose studied. Drug exposure at high doses were 6-9 times in mice and 7-33 times in rats the human exposure at 20 mg. In dogs, there was perivascular inflammation in the lungs in 3/6 dogs vs. 0/6 in controls at approximately the same exposure as men taking 20 mg in the 1-month study. In a 6-month study, drug-induced exacerbation of Beagle Pain Syndrome associated with both acute- and chronic disseminated arteritis was observed in both control and treated groups with increased incidence and severity at the high dose. However, the lowest free drug concentration in an affected dog was 29 times greater than the free drug concentration in men taking 20 mg. The vasculitis findings were not seen in the 1-year dog study with doses giving exposures up to 33 times the human exposure. Instead, marked thrombocytopenia/neutropenia indicative of type III immunopathy were seen in 1 mid- and 1 high dose female with 14 and 18X the human exposure. The one high dose female also had a single focus of left coronary artery perivasculitis. Hypersensitivity is the major manifestation of clinical drug-induced vasculitis, which is represented by anemia, leukopenia, thrombocytopenia, vasculitis, *etc.* Possible systemic symptoms of hypersensitivity such as myalgia, back pain or infection were the most frequently reported adverse human events associated with IC351.

In dogs, a therapeutic index could not be determined since the exposure for the desired pharmacological activity and the vasculitis was not determined. However, the unbound drug exposure in dogs at a NOAEL was 1- to 3 fold at 10 mg/kg in the 1-month study and 3- to 33 fold at 60 mg/kg/day in the 6-month study (due to individual variability) the exposure of men taking 20 mg. One Phase II clinical study (LVBF) evaluated erythrocyte sedimentation rate (ESR) and serum creatine kinase (CK). There was lack of association found between back pain or myalgia and either increased ESR or serum CK. Sponsor concluded that neither back pain nor myalgia was associated with inflammatory or myopathic etiologies. Additional information from skin biopsies, evaluation of deposition of immune complexes in vessels or plasma/serum elevations in the cytokines/ANCA may be helpful to monitor the hypersensitivity vasculitis.

Recommendations: The preclinical data support the safety of the proposed dose of 20 mg of Cialis.

X. APPENDIX/ATTACHMENTS:

Appendix I: IND 54,553 Reviews 1/2/3

Appendix II: CAC minutes for protocol and final studies
Appendix III: 2-Year carcinogenicity survival rate
Appendix IV: 2-Year carcinogenicity body weight changes versus dose level
2-Year carcinogenicity group body weight summary
2-Year carcinogenicity individual data listing
Appendix V: 2-Year carcinogenicity sponsor's histopathological incidence tables
Appendix VI: Statistical review and evaluation from the agency

Addendum to review: See next page

Any compliance issues:

Addendum to Review

4/12/02
NDA: 21-368
Drug name: Tadalafil
Sponsor: Lilly ICOS LLC
Division: DRUDP, HFD-580
Reviewer: Yangmee Shin

Review of Total Radioactivity for Carcinogenicity Study Doses

Sponsor submitted a summary of the data for saturation of total drug-related substances associated with IC351 by measuring the total radioactivity exposure after oral administration of [^{14}C]-IC351 (specific activity) in male rats, and male and female mice. The animals were administered daily doses of 400, 800 and 1000 mg/kg formulated as the IC351:HPMCP at least for 2 weeks followed by a single dose of [^{14}C]-IC351:HPMCP.

Results:

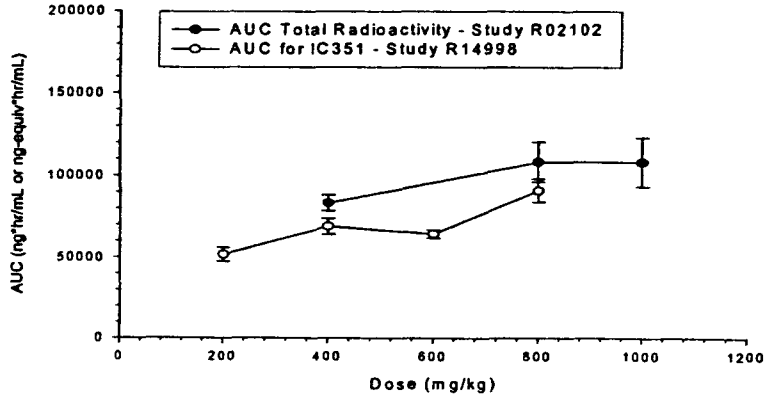
Rats (R02102);
A 2-fold increase in dose from 400- to 800 mg/kg resulted in a 1.3-fold increase in total radioactivity exposure (AUC), and no further increase in exposure at 1000 mg/kg in male rats, suggesting subproportional increases in exposure with increasing dose.

Table 1: Plasma PK Parameters of Total Radioactivity on Day 14 Following 2 Weeks of Daily Oral Administration of 400, 800 or 1000 mg IC351/kg/day to Male Wistar Rats

Parameter	Dose (mg/kg/day) [a]		
	400	800	1000
$AUC_{0\text{-}24hr}$ (ng-equiv*hr/mL)	82973	108068	108034
AUCSEM	4842	12048	14985
C_{max} (ng-equiv/mL)			
T_{max} (hr)	8	4	4

[a] [^{14}C]IC351:HPMCP was administered on the final day of dosing and plasma radioequivalent concentrations were measured after this dose.

Abbreviations: AUC = Area under the plasma concentration time curve between 0 and 24 hours; AUCSEM = standard error estimate of the variability of mean AUC; Cmax = Maximal observed plasma concentration; T_{max} = time to reach maximal plasma concentration.



Mouse;

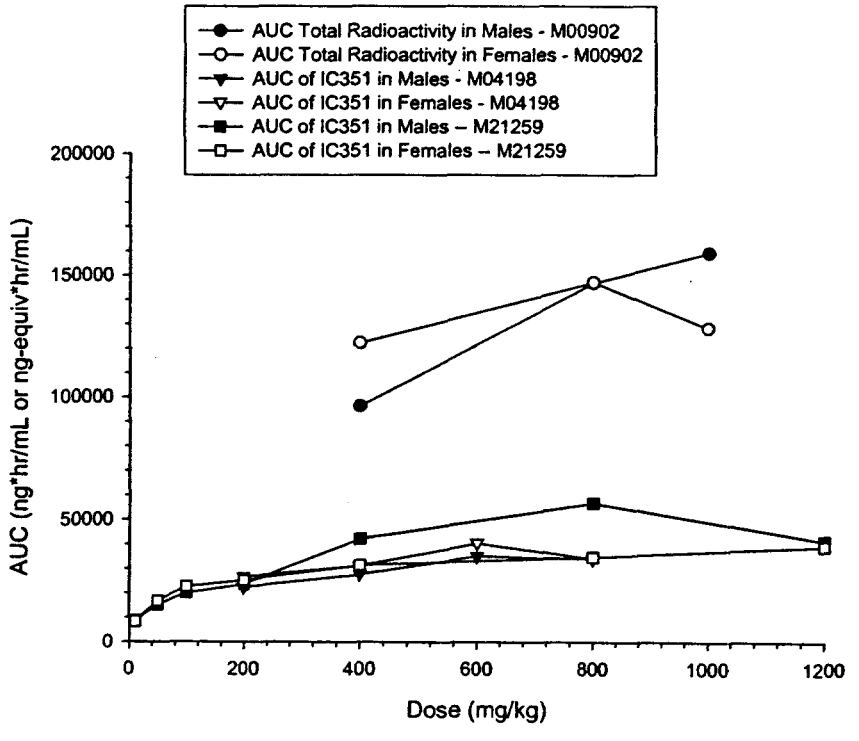
A 2-fold increase in dose from 400- to 800 mg/kg resulted in a 1.5-fold increase in total radioactivity exposure (AUC) in male mice and a 1.2-fold increase in female mice, and 1.1-fold increase in exposure in male mice and 1.1-fold decrease in female mice at 1000 mg/kg. The data suggest that the increase was subproportional to dose. The apparent differences between male and female exposures were considered to be normal study-to-study variability since the radioequivalent concentrations after a single dose (000M02) was different than on Day 16 in the present study. In a pilot PK study in mice with a single dose of 400 mg/kg (000M02), plasma concentration vs. time profile of total radioactivity corresponded to the profile for unchanged IC351, indicating that absorption of IC351 would be expected to govern metabolite disposition. The methylcatechol glucuronide demonstrated formation rate limited PK, as the half-life values were similar for IC351 and the metabolite.

Table 2: Plasma PK Parameters of Total Radioactivity on Day 16 following 2 Weeks of Daily Oral Administration of 400, 800 or 1000 mg IC351/kg/day to CD-1 Mice

Parameter	Dose (mg/kg/day) [a]					
	Male			Female		
	400	800	1000	400	800	1000
Day 16						
$AUC_{0\text{-}24hr}$ (ng-equiv*hr/mL)	96170	146784	159160	122117	147103	128247
C_{max} (ng-equiv/mL)						
T_{max} (hr)	4	6	2	2	2	1

[a] [^{14}C]IC351:HPMCP was administered on the final day of dosing and plasma radioequivalent concentrations were measured after this dose

Abbreviations: AUC = Area under the plasma concentration time curve between 0 and 24 hours; C_{max} = Maximal observed plasma concentration; T_{max} = time to reach maximal plasma concentration.



Conclusion:
In male rats, there was a 30% increase in total radioactivity when the dose was raised from 400 mg/kg to 800 and 1000 mg/kg. In female mice, the increase was 20% at 800 mg/kg and 5% at 1000 mg/kg. Clearly, saturation of absorption was achieved at 400 mg/kg in these two cases. In male mice, doubling the dose from 400 to 800 mg/kg increase total radioactivity 53% and increasing the dose to 1000 mg/kg increased total radioactivity 65%. Although saturation of absorption was not achieved, the increase is clearly non-proportional to dose and is reaching saturation.

Recommendation:
Three of four arms of the two carcinogenicity studies (male and female rats and female mice) are clearly valid. Also, saturation of absorption may have been achieved in male mice since no gender difference in exposure to IC351 has been seen in previous studies in mice and the apparent differences between male and female exposures in the present study may be within the normal study-to-study variability. Furthermore, at 400 mg/kg, the AUC in male mice is 10 times higher than in men taking 20 mg daily. Considering all the data, the carcinogenicity studies for IC351 are acceptable. No additional carcinogenicity studies are necessary.

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/s/

Yangmee Shin
4/22/02 04:25:24 PM
PHARMACOLOGIST

Alexander W. Jordan
4/29/02 07:39:30 AM
PHARMACOLOGIST

To: Florence Houn
Director ODE III

From: John Leighton
Associate Director for Pharmacology/Toxicology, ODE III

Subject: NDA 21-368
Cialis (tadalafil)

Date: April 23, 2002

Introduction

Lilly ICOS is seeking approval for Cialis for treatment of erectile dysfunction (ED). The sponsor has conducted pharmacology, safety pharmacology and toxicology studies, including 6-month studies in rats and 12 month studies in beagle dogs. Studies to investigate the reproductive toxicity, genotoxicity and carcinogenicity of tadalafil have also been conducted. The Division review of pharmacology and toxicology data submitted by the sponsor support the safety of the proposed 20 mg dose of Cialis

Review of Draft Pharmacology/Toxicology Safety Issues

The Division Pharmacology/Toxicology review noted two outstanding issues to be addressed. These include vasculitis observed toxicology studies in mice, rats and dogs, and a recommendation for an additional alternative mouse carcinogenicity assay for a Phase 4 commitment. This commitment would not be necessary if the sponsor provided additional evidence for saturation of absorption.

The Division Pharmacology/Toxicology proposes addressing the issue of vasculitis observed in animal studies through information in the label. The Division extensively considered the animal findings and their relevance to clinical use of Cialis. I concur with the Division's analysis but recommend that the Division seek the opinion of the Pharmacology/Toxicology Coordinating Committee (PTCC) or appropriate subcommittee to ensure consistent labeling of these findings.

The sponsor provided additional information that demonstrates saturation of absorption at high dose in their carcinogenicity studies. The Executive CAC members participating in the original review concurred with this conclusion. Therefore, the additional alternative mouse carcinogenicity assay is not necessary, and Cialis should be considered negative as tested in the mouse and rat carcinogenicity assays.

No additional pharmacology or toxicology issues remain to be addressed.

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/s/

John Leighton
4/24/02 04:24:58 PM
PHARMACOLOGIST

APPENDIX I

IND 54,553 Reviews 1, 2, 3

IND 54,553

May 26, 1998

Div of Reproductive and Urologic Drug Products, HFD-580

Jeri El-Hage, Ph.D.

Submission: # 000, November 6, 1997

Review of Pharmacology and Toxicology Data
Review # 1

Drug: IC351, (6R-trans)-6-(1,3-benzodioxol-5-yl)2,3,6,7,12,12a, hexahydro-2-methyl-pyrazino[1,2:1,6]pyrido[3,4-b]indole-1,4-dione, Drug substance [redacted]

CAS Number : 171596-29-5

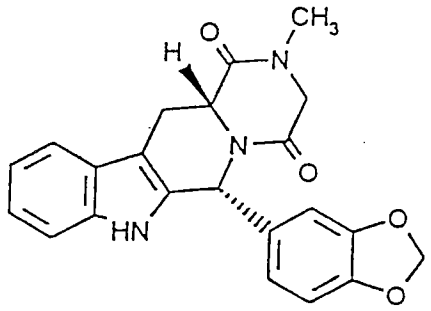
Sponsor: ICOS Corporation, Bothell, WA

Manufacturer:

Drug substance [redacted]

Drug product:

Structure:



Molecular Wt. = 389.41 **Molecular formula** = $C_{22}H_{19}N_3O_4$

Drug Class: Beta-carboline Phosphodiesterase type 5 inhibitor

Indication: Erectile Dysfunction

Clinical formulation: Tablets containing 5 or 25 mg IC351 and hydroxypropylmethylcellulose phthalate (1:1) with numerous inactive ingredients including microcrystalline cellulose, croscarmellose sodium, sodium lauryl sulphate, povidone D30 and magnesium stearate and Opadry film coat. The qualitative composition of the tablets is summarized in the table below.

Table 3. Quantitative Composition of IC351 Tablets

Component	Formulations (mg per tablet)
1. IC351, co-precipitate	[redacted]
2. Microcrystalline cellulose	[redacted]
3. Croscarmellose Sodium	
4. Sodium Lauryl Sulphate	
5. [redacted]	
6. Purified Water, USP (Water for Irrigation)	[redacted]
7. Croscarmellose Sodium	
8. Sodium Lauryl Sulphate	[redacted]
9. [redacted]	
10. Magnesium Stearate	
Tablet core subtotal	[redacted]

Clinical Protocol: Phase II DB, PC Safety, Efficacy and Dose-Response Study in Men with Mild to Moderate Erectile Dysfunction (n = 300 subjects). The safety, tolerability, efficacy and dose-response relationship of 0, 10, 25, 50 and 100 mg oral doses of IC351 administered once daily for 21 days will be assessed.

Non- US Studies Planned:
Protocol DSD02 (Netherlands)- DB, PC study to evaluate the safety, tolerability, and pharmacokinetics of IC351 in healthy elderly men after single and multiple doses.

Protocol DSD01 (Netherlands) - DB, PC single dose crossover study to assess efficacy of a 100 mg IC351 in producing erections (Rigiscan evaluation).

Previous Human Experience: Three Phase I studies were conducted by [redacted]

Protocol [redacted] (U.K.) - Four groups of healthy male subjects (n= 4/group) received placebo and three escalating doses of IC 351 in a four-way crossover study. The dose groups were as follows: Group 1- 1, 2 and 5 mg; Group 2- 5, 10 and 25 mg; Group 3 25, 50 and 200 mg; Group 4 100, 250 and 500 mg.

Protocol [redacted] (U.K.) Effect of Food and Bioavailability Study in Healthy Male Subjects (n = 13). The study was a 3-way crossover study with subjects taking 100 mg in an oral suspension, two 50 mg tablets while fasting or two 50 mg tablets after a standard meal. Food significantly increased drug absorption (1.5 to 2 fold). 44 adverse events were reported with headache as the most common AR (22 reports).

Severe headache and backache (lasting 3-4 days) were observed in 3/13 subjects.

Protocol (Germany) DB, PC study to compare safety, tolerability and pharmacokinetics of IC351 in young and elderly volunteers after 8 days of dosing with 50 mg/day. Fourteen young (18-40 years) subjects completed 8 days of dosing. The study was suspended due to concerns regarding the ECG changes and vasculitis observed in the preclinical studies. None of the elderly volunteers were dosed.

PHARMACOLOGY

In vitro studies with IC351 in cultured cells have demonstrated potent competitive and reversible inhibition of cGMP-specific type 5 phosphodiesterase (IC_{50} = 2.5nM with bovine aorta and recombinant human PDE 5; K_i = 4.5nM). Tissue source for the rhPDE 5 was not specified. The selectivity of IC351 for the various phosphodiesterases are summarized in the table below.

Table 2. Selectivity of IC351 for PDE5

	PDE1	PDE2	PDE3	PDE4	PDE5	PDE6	PDE7
% inhib. @ 10 µM	10-40	0-24	16	24	99	86	0
IC 50 (µM)	>10	>10	>10	>10	0.0025	3.4	>10

In cultured cells (vascular smooth muscle, renal epithelium, human umbilical vein endothelium), IC351 increases basal levels of cGMP (4-fold) and greatly potentiates cGMP levels produced by the guanylyl cyclase activating agents atrial natriuretic factor, C-type natriuretic peptide, and sodium nitroprusside(4 to 200 fold). IC351 had no effect on cAMP levels in treated cells. IC351 had no direct effect on platelet aggregation but potentiated the inhibitory effect of sodium nitroprusside.

IC351 had no effect on phenylephrine-precontracted aortic rings which were endothelium denuded (i.e., lacking nitric oxide synthetase). In phenylephrine precontracted aortic rings with intact endothelium, IC351 produced dose-dependent relaxation. IC351 was believed to potentiate the effect of endogenous nitrous oxide. IC351 did not alter the contractile properties of rat papillary muscle. IC351 induced significant long-lasting reductions in blood pressure in 3 hypertensive rat models, namely, SHR, DOCA-salt, and renal hypertensive rats. IC351 also reduced mean blood pressure in normotensive rats. Effects of IC351 on mean blood pressure in rats are summarized in the table below taken directly from the submission (vol 1.2, p 100. IC351 (0.1 to 3 mg/kg) produced dose-dependent decreases

in blood pressure (10-12%) in anesthetized beagle dogs secondary to decreased vascular resistance.

Table 1. The Effects of Orally-Administered IC351 on Mean Arterial Blood Pressure in Hypotensive and Normotensive Rats

Exp	Animal Model	Dose (mg/kg)	No. of Animals		Initial MAP	AUC MAP 0-7h	ΔMAP_{max}	Effect at 7h
			Control	Test	(mmHg)	(mmHg.h)	(mmHg)(time)	(mmHg)
1		5	8	8	185±3	171±38	-32 (2h 30)	-18±11
2		5	10	7	178±4	171±28	-27 (1h 30)	-23±7
3		1	8	7	190±4	164±43	-26 (2h 30)	-18±9
4		1	10	9	179±5	90±30	-18 (2h 30)	-11±8
5		5	5	5	166±5	306±72	-51 (4h)	-39±15
6		1	5	8	173±6	223±53	-47 (3h 30)	-34±13
7		5	4	7	185±10	149±61	-23 (2h)	-25±9.9
8		5	5	7	128±5	72±53	-20 (3h)	2±22

Safety Pharmacology

Oral doses of 10, 30 and 100 mg/kg were administered to conscious rats (n=3 males/dose) and dogs (n = 1/sex/dose). Vomiting was observed in ½ dogs at all dose levels. Increased water consumption was observed in rats and dogs for several hours after dosing.

Cardiovascular Effects- Moderate tachycardia (20-40 bpm) was observed in dogs at doses ≥ 30 mg/kg. The effective antihypertensive dose of IC351 in the rat is 1 mg/kg,po. The effects of IC351 on blood pressure (BP) in various rat models is summarized in the table above. In conscious dogs, single oral doses of 20 and 200 mg/kg IC351 produced slight reductions in mean blood pressure without effects on heart rate or respiration rate.

CNS Effects- Administration of 2, 20 or 200 mg/kg,po to conscious rats and dogs had no effects except to produced moderate ptosis in 2/3 HD rats. IC351 at doses of 10, 30 and 100 mg/kg,po had no effect on pentobarbitone sleeping time in mice.

Renal Effects- Administration of IC351 (0.3 mg/kg,iv) produced significant diuresis and sodium excretion in rats. Potassium excretion was unaffected. IC351 (0.1 mg/kg,iv) also potentiated atrial natriuretic factor-induced diuresis and natriuresis.

Absorption, Distribution, Metabolism and Excretion (ADME)

A summary of studies performed, vehicles used and results was taken directly from the submission(vol 1.2) and are attached in appendix I. IC351 is rapidly absorbed in rats and dogs with an oral bioavailability of 53% in the rat and 34% in dogs. Increases in plasma concentration are not seen at oral doses greater than 400 mg/kg/day in males and 800 mg/kg/day in females due to a saturation of absorption. Plasma levels in females are generally higher than in males. The tables below summarize the absorption profiles of IC351 in rats and dogs (taken from vol 1.3 studies R21147 and D21148).

Toxicokinetics Data of ICI351 in Han Wistar Rats(R21147)

Summary of toxicokinetic data

Dosage (a) (mg/kg)	Day of dosing	Sex	C_{max} (ng/mL)	t_{max} (h)	$AUC_{(1-24h)}$ (ng.h/mL)
50	1	M	3210	4	37400
		F	3670	4	45800
100	4	M	5160	4	66000
		F	5680	4	70900
200	6	M	5190	4	77000
		F	5760	8	93600
400	8	M	8370	4	145000
		F	9910	2	152000
800	11	M	8500	8	140000
		F	11600	4	248000
1200	13	M	7830	4	147000
		F	12100	6	NC

(a) Expressed as [redacted]

NC Not calculated due to absence of data for 24 hours after dosing.

Toxicokinetics Data for IC351 in Beagle Dogs (D21148)

Summary of mean toxicokinetic data (D21148)

Dosage (mg/kg)	Day of dosing*	Sex	Mean C_{max} (ng/mL)	t_{max} range (h)	AUC_{0-24h} (ng.h/mL)
50	1	M	2720	2-8	43600
		F	4040	8-24	64500
100	3	M	4220	1-2	76800
		F	6160	2-8	11500
200	6	M	4800	2-4	90200
		F	7410	2-8	157000
400	8	M	7850	4	134000
		F	8400	2-8	175000
600	10	M	7530	4-6	132000
		F	11100	2-4	204000

* - First day of treatment was designated Day 0

The pharmacokinetics of IC351 in Wistar rats were determined after single intravenous or oral doses of 10 mg/kg (study BPW662). IC351 was measured by HPLC [redacted]. Kinetics data are summarized in the table below.

	Intravenous		Oral	
IC 351	Male	Female	Male	Female
Cmax, ng/ml	3700	5670	788	856
Tmax, hours	0.08	0.08	1 hr	2 hrs
AUC 0-24, ng.hr/ml	17981	36189	9591	12335
AUC 0-∞, ng.hr/ml	18064	36659		
kel, hours	0.218	0.167		
Half-life, hours	3.2	4.2		
Clearance, ml/min/kg	8.9	4.4		
Volume of distrib, L/kg	2.5	1.6		
Dose in urine, %	0.33	0.47		
Oral bioavailability, %			53	34
Drug-related radioactiv.				
Cmax, ng/ml	5744	7200	1051	1177
Tmax	0.08	0.08	1 hr	2 hrs
AUC 0-72, ng.h/ml	34319	51307	22281	24210

IC351 has a low plasma clearance and a moderate volume of distribution. Renal clearance is negligible in the rat (<1% of dose excreted in urine unchanged). Drug absorption after oral dosing is prolonged and incomplete. Measurement of total radioactivity revealed a significant proportion of radioactivity (40-50%) is not attributable to parent compound.

Identification of IC351 Metabolites Excreted in Rat and Dog Bile

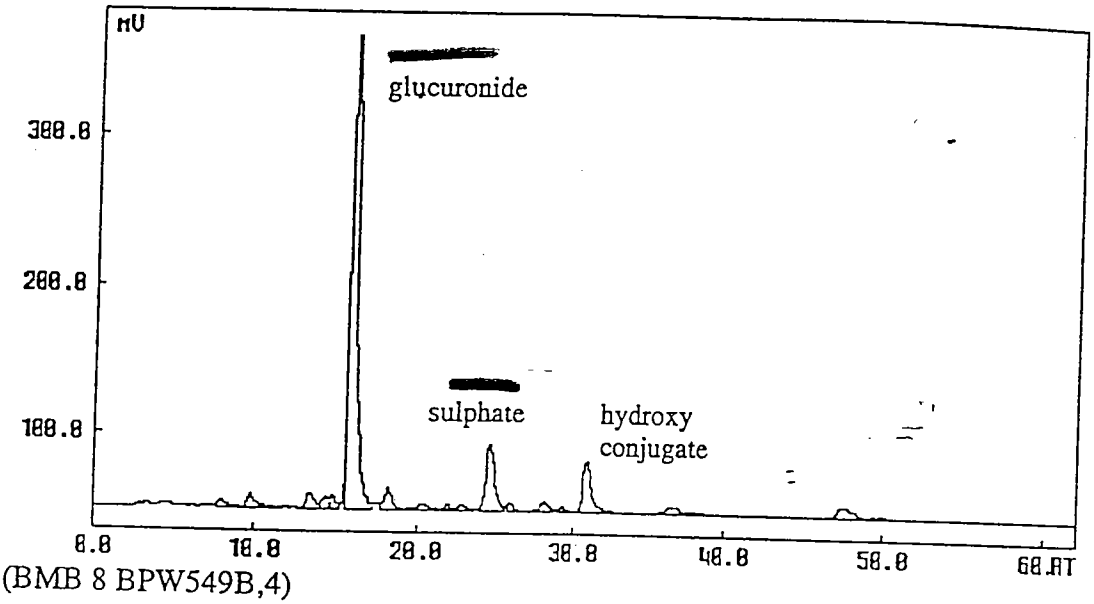
Male Wistar rats (n=3) and one beagle dog were administered 3 mg/kg of C^{14}-IC351, intravenously and bile was collected hourly for 4 hours in rats and 8 hours in dogs. A collective 4-24 hour sample was also collected for rats. The dog data were not adequate. Only 6% of the dose was recovered in the 8 hour collection period and the urine was contaminated with bile, suggesting a leak in the collection assembly. Metabolite profiles in bile of the dog and rat are presented in Figures 1 and 2 taken directly form the submission (vol 1.3, pp 489-490).

IND 54,55
p. 8

FIGURE 1

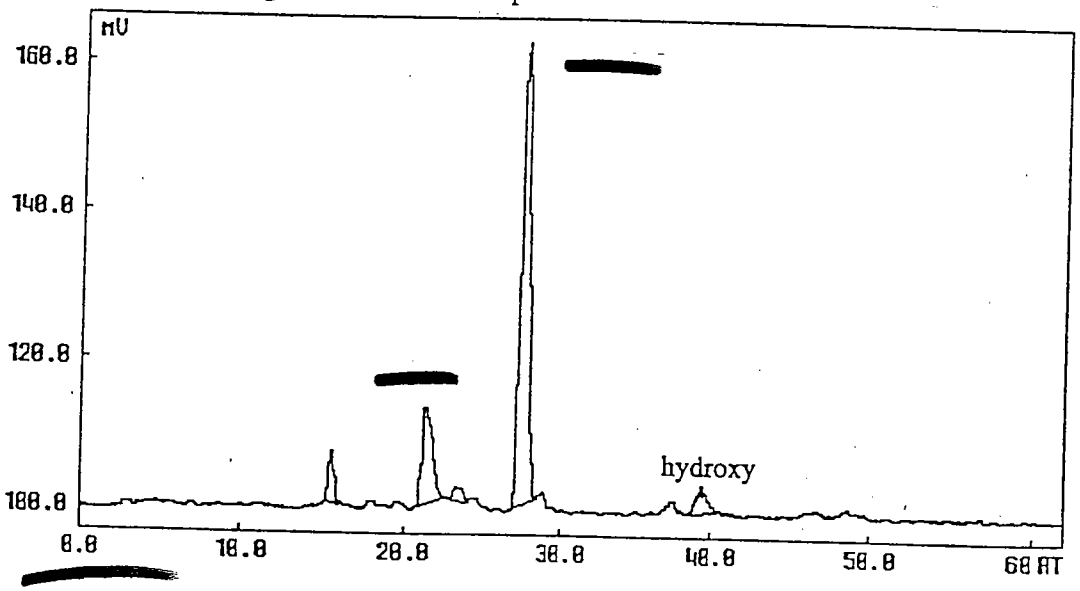
METABOLITE PROFILE IN BILE AND HYDROLYSED BILE AFTER INTRAVENOUS ADMINISTATION OF ^{14}C- [redacted] TO THE DOG (3MG/KG)

Non-hydrolysed (0-8h pooled dog bile . [redacted]



(BMB 8 BPW549B,4)

92h hydrolysis with glucuronidase + sulphatase



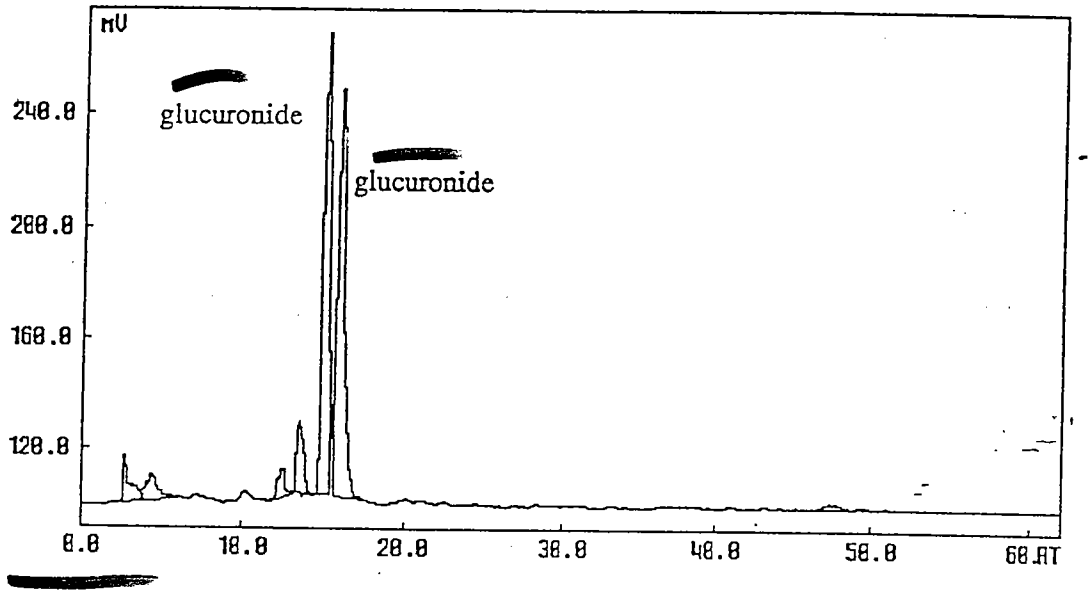
[redacted]

[redacted] IND 54,553
P. 78

FIGURE 2

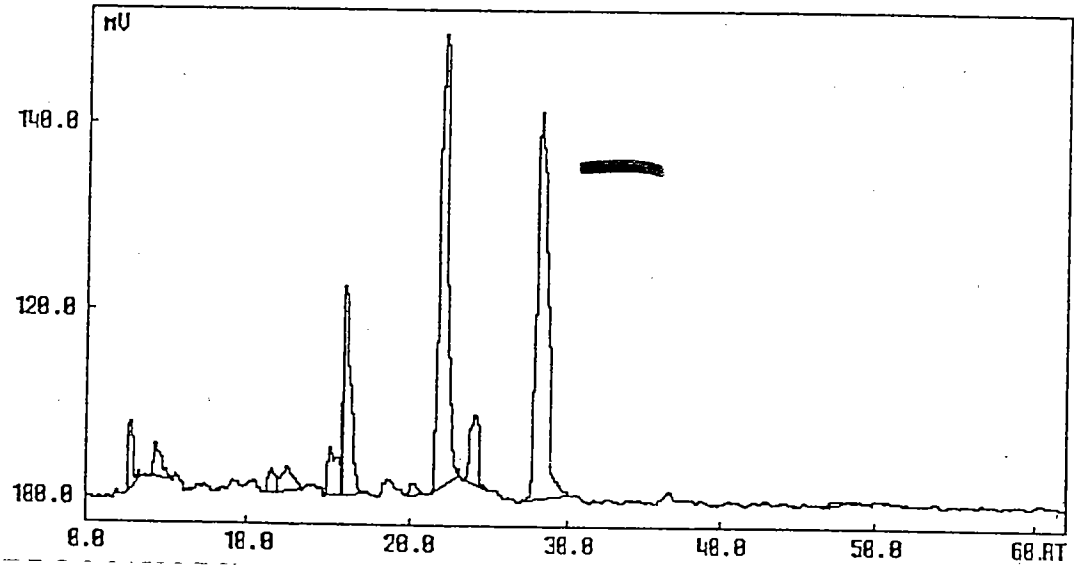
METABOLITE PROFILE IN BILE AND HYDROLYSED BILE AFTER INTRAVENOUS ADMINISTATION OF ^{14}C- [redacted] TO THE RAT (3MG/KG)

Non hydrolysed (0-24h pooled rat bile [redacted])



[redacted]

67h hydrolysis with glucuronidase + sulphatase

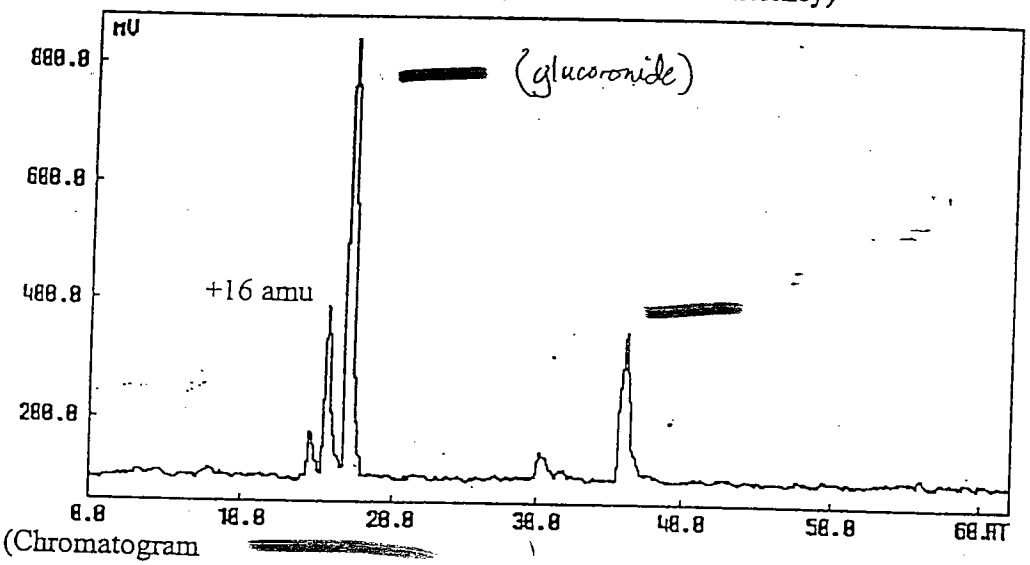


[redacted]

FIGURE 2

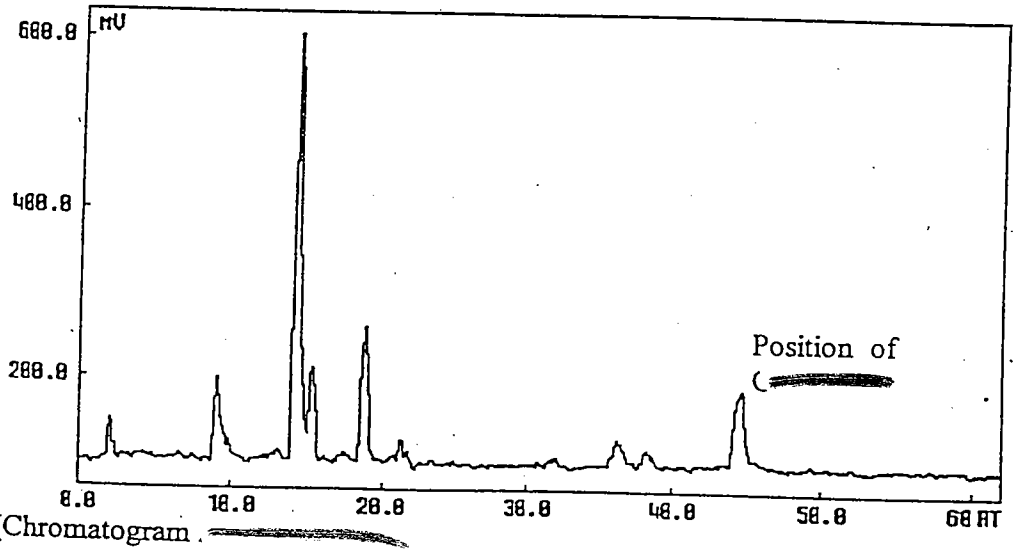
METABOLITE PROFILE OF A 24-48 HOUR FAECAL SAMPLE EXTRACT AND A 0-24 HOUR URINE SAMPLE OBTAINED FROM THE HAN WISTAR (HW) RAT AFTER SINGLE ORAL ADMINISTRATION OF ^{14}C- [redacted] AT A DOSE-LEVEL OF 10MG/KG BODYWEIGHT

24-48 hour faecal extract
(female rat 447307, 82% dose in faeces, 86% extraction efficiency)



(Chromatogram [redacted])

0-24 hour urine sample
(female rat 447307, 3% dose in urine)



(Chromatogram [redacted])

[redacted]

Distribution of IC351 in the Han Wistar Rat After Single IV or Oral Dosing (study BPW618)

Four male rats were dosed with 10 mg/kg C^{14}-IC351 orally and 1 animal/timepoint was killed at 1, 6, 24 and 168 hours. An additional 3 animals/sex were dosed for an excretion study. Excretion was primarily fecal (98% in male, 97% in female rats). Urinary excretion occurred within the first 24 hours, fecal recovery was more prolonged with 23% recovery within 24 hours and 71% recovery within 24-48 hours. [redacted] was the i [redacted] The levels of radioactivity in tissues after single oral doses are summarized in the table below. One hour after dosing, radioactivity was concentrated in the stomach, GI contents, lungs > thyroid > adrenal > liver, kidney > blood. At 6 hours highest concentrations were observed in the GI contents, stomach wall > adrenal, intestinal wall, liver, pancreas, kidney > blood. At 24 hours the adrenals, intestines, liver, pancreas and stomach still had concentrations of radioactivity 5-fold higher than blood.

Concentration (µg/g)

Animals :	447298	447299	447300	447301
Time (h):	1	6	24	168
Tissues:				
Adrenals	2.53	3.19	2.35	<0.34
Bladder contents	NS	NS	NS	<0.34
Bladder wall	NS	NS	NS	<0.34
Blood	<0.08	0.33	0.43	<0.34
Bone	<0.08	0.46	<0.15	<0.34
Brain	0.12	<0.08	<0.15	<0.34
Brown fat	0.59	0.68	0.68	<0.34
Gi contents 1	44.55	52.18	>20.3	<0.34
Gi contents 2	27.63	29.77	13.93	<0.34
Gonads	0.17	0.55	0.18	<0.34
Harderian gland	NS	0.50	0.71	<0.34
Heart	0.24	0.64	0.67	<0.34
Kidney	0.56	1.14	0.80	<0.34
Large intestine wall	NS	2.47	2.47	<0.34
Liver	0.86	1.89	2.39	0.36
Lung	28.11	0.48	0.55	<0.34
Muscle	0.18	0.55	0.48	<0.34
Pancreas	0.25	1.61	2.99	<0.34
Pituitary	<0.08	0.50	0.38	<0.34
Red bone marrow	<0.08	0.50	0.33	<0.34
Salivary glands	0.46	0.52	0.56	<0.34
Small intestine wall	NS	2.50	2.07	<0.34
Spleen	0.55	0.71	0.58	<0.34
Stomach contents	32.27	>70.0	>20.3	<0.34
Stomach wall	36.24	25.63	7.08	<0.34
Thymus	0.14	0.49	0.23	<0.34
Thyroid	14.00	NS	<0.15	<0.34

Pharmacokinetics,Metabolism and Excretion of IC 351 in Beagle Dogs (studies BPW641, BPW659)

Beagle dogs(n = 3/sex) were administered 10 mg/kg C^{14}-IC351 intravenously or orally and pharmacokinetics parameters were determined. Data are summarized below in a table taken directly from the submission (vol 1.3, p.525).

Concentrations of [redacted] were determined by HPLC [redacted] following [redacted] Levels of total radioactive drug-related material were determined by liquid scintillation counting. The following pharmacokinetic parameters were obtained:-

Intravenous [redacted]		Male dogs 3IC5	4CG3	4IF3	Mean	Female dogs 4HD2	4HF6	4HP2	Mean
Cmax	ng/mL	3010	5080	2900	3663	5280	4070	4110	4487
tmax	h	0.08	0.33	0.08	0.16	0.08	0.08	0.08	0.08
AUC_{0-24}	h.ng/mL	18103	24658	25921	22894	25001	24653	22959	24204
AUC_{∞}	h.ng/mL	18257	24712	27626	23532	25045	25494	23577	24705
t1/2	h	3.4	2.7	6.1	4	2.6	5	4.7	4
Clp	mL/min/kg	8.2	5.8	5.4	6	6	5.7	6.3	6
Vd (area)	L/Kg	2.4	1.3	2.8	2	1.3	2.5	2.6	2
Dose in urine	%	0.1	0.1	0.1	0.1	0.1	0.3	0.3	0.2
Radioactive drug-related material									
Cmax	ng/mL	3852	6853	3767	4824	6996	5812	5267	6025
tmax	h	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08
AUC_{0-168}	h.ng/mL	51130	80624	64201	65318	84702	122278	94265	100415
AUC_{∞}	h.ng/mL	59629	81795	68273	69899	86284	124657	95683	102208
Oral									
[redacted]									
Cmax	ng/mL	468	279	832	526	180	535	533	416
tmax	h	4	0.67	4	3	0.67	72	6	26
AUC_{0-24}	h.ng/mL	3884	1086	7357	4109	942	1409	4482	2278
F_{0-24}	%	21	4	28	18	4	6	20	10
t1/2	h			3				2.7	
Radioactive drug-related material									
Cmax	ng/mL	1158	730	1374	1087	643	1627	1604	1291
tmax	h	4	3	4	4	4	72	6	27
AUC_{0-146}	h.ng/mL	24054	26466	22614	24378	42474	99941	44586	62334
AUC_{∞}	h.ng/mL	24137	26574	22766	24492	43417	100069	44994	62827

Plasma Protein Binding of IC351 (studies BPW 770,BPW 507)

Protein binding of IC351 was similar over the concentration range of 12 to 1200 ng/ml, indicating no saturation of protein binding. IC351 was 85% bound to albumin, 90% bound to α1-acid glycoprotein, 15% bound to gamma globulins and 96% bound in the presence of a mixture of all 3 plasma proteins.
Binding of C^{14}-IC351 was determined by in vitro equilibrium dialysis over a concentration range of 10 to 10,000 ng/ml. Plasma protein binding was 92% in rats, 87% in dogs, and 94% in human plasma.
The distribution pattern of C^{14}-IC351 (40 to 10,000 ng/ml) in rat, dog and human blood was examined. IC351 had a slightly greater affinity for plasma than whole blood with ratios of 1.21, 1.21, and 1.39 in rat, dog, and humans, respectively.

TOXICOLOGY

Acute Toxicity

Study Number	Species	Dose mg/kg/ Route	Observations
M20798	Mouse, B6C3F1 3/sex/dose	400, 650, 1000 1600, 2000 po	Lethal dose > 2000 mg/kg No signs or necropsy findings
M20799	Mouse, B6C3F1 10/sex	0 or 2000, po	5/sex killed on day 3 or day 14. No deaths, signs, or pathology.
M20977	Mouse, B6C3F1 3/sex/dose	37.5, 62.5, or 100, iv	1 vehicle and 4/6 HD mice died. PEG400 vehicle/dose volume contributed to toxicity. Prostration, labored breathing with ≥ 62.5 mg/kg
M20978	Mouse, B6C3F1 10/sex/dose	0 or 62.5, iv	Prostration, tremor, labored breathing after dosing with IC351. No mortality.
R20796	Rat, Wistar 3/sex/dose	400, 650, 1000 1600, 2000, po	Lethal dose > 2000 mg/kg. Vocalization in 3 HD females. Croaking in 1M, 1 F @ 1600. 3M @ 1000 -subdued; 1M @ 1000 prostrate, labored breathing.
R20797	Rat, Wistar 10/sex	0 or 2000, po	No mortality or signs.
R20979	Rat, Wistar 3/sex/does	0, 37.5, 62.5, iv	No mortality. Subdued behavior, rapid breathing, prostration, piloerection all groups. Some HD rats were moribund and had convulsions.
R20980	Rat, Wistar 10/sex	0 or 37.5, iv	1 HD female died. Signs included subdued behavior, jerky movement, labored breathing.

Maximum Repeatable Daily Oral Dose Study in Rats (R20791)

Han Wistar rats (n = 6/sex) were dosed with 100(days 0,1), 200 (days 2-4), 400(days 5-6), 800(days 7-8), 1400(days 9-11) and 2000 (days 12-18) mg/kg/day IC351 with each dose given 2 to 3 days.

There were no deaths. Clinical signs were noted primarily at 2000 mg/kg and were secondary to the large dose volume. Signs included reflux of dose, noisy breathing, vocalizing, and salivation. There was no drug-related pathology. The toxicokinetics data are presented in the table below taken directly from the submission (vol 1.5, p 1326). The sponsor concludes there is saturation of absorption at doses > 400 mg/kg and therefore, selected 400 mg/kg as the high dose for the one month oral toxicity study in rats. Cmax levels plateau at doses > 400, however, AUC continues to increase with doses ≥ 1400 mg/kg, albeit the increases are less than dose proportional.

Group	Dosage (mg/kg)	Day of dosing	C_{max} (ng/mL)		t_{max} (h)		$AUC_{(1-24h)}$ (ng.h/mL)	
			Male	Female	Male	Female	Male	Female
A	100	0	4200	5780	4	8	51410	~94260
	200	2	3900	5090	2	8	44180	76170
	400	5	5730	7810	4	2	52450	107300
	800	7	4900	8140	2	2	44810	78660
	1400	9	4330	8480	4	1	55740	146300
	2000	12	5220	8290	4	2	81000	125700
	2000	18	6140	9290	4	1	83850	134500
B	10	21	540	1190	2	4	4240	14650

Conclusion-Repeat dose toxicity studies must be evaluated to determine if the maximum dose selection was acceptable.

<u>28-Day Oral Toxicity Study in Han Wistar Rats (R20861, lot # C2856/121/1)</u>

The study was conducted by [redacted] The study was conducted according to GLP.

Han Wistar rats (n = 12/sex/dose) were administered 0, 10, 60 or 400 mg/kg/day IC351 orally by gavage in a solution of 0.5% hydroxypropylmethylcellulose(HPMC) and 1% Tween 80 in sterile water. An additional 8 rats/sex were dosed with vehicle or 400 mg/kg for 28 days and maintained for a 21 day drug-free recovery period.

Mortality- One control female killed in extremis secondary to a gavage accident.

Clinical signs- The sponsor reports incidental observations of pawing cage floor in males; hunched posture and thin appearance in females, reflux of dose, and hair loss. Data were not provided.

Body weight/food consumption- No effects in males, slight increase in mean body weight in high dose females. No changes in food consumption.

Ophthalmology- sponsor reports no drug effects. Data not provided.

Hearing tests- rats were exposed to a 54 and 74 dB sound and presence of pinna reflex was assessed. Sponsor states there were no drug-related effects on hearing.

Hematology (days 9, 21, 43)-
Reticulocyte counts- increased in all IC351 treated groups on day 9- 20% in males, 40% in females.
Mean cell volume- mildly increased in all treated males, day 21
WBC- increased in high dose females days 9, 21
Total leukocyte counts/Lymphocytes- increased in MD, HD females (HD day 9, MD,HD day 21)
Monocytes- increased in HD females, days 9, 21
Thrombotest clotting time- increased in all treated males, LD, HD females day 21 (reersed after drug free recovery).
Hematology changes were reversible after 3 week recovery period.

Clinical chemistry (days 9, 21, 41)
Alkaline phosphatase - decreased in all treated males day 9
Bilirubin - decreased in MD, HD males day 9; decreased in all treated females day 21.
Urea nitrogen - increased at all dose levels in males, day 9
Total protein - decreased at all dose levels in males and females, days 9 and 21
Albumin- decreased in MD, HD males and females on days 9 and 21
Cholesterol- decreased(25%) at all doses in males, days 9 and 21
Potassium- increased in HD females, day 21
Inorganic Phosphate - increased in MD, HD females day 21.
Changes in clinical chemistry parameters were reversible, returning to normal after the 3 week recovery period.

Urinalysis (days 23,43)- Urinary sodium and chloride excretion were increased in HD females on day 23.

Organ weights -(only values for relative weights were provided)
Heart- increased in HD males
Lungs- increased in all treated males
Thymus- decreased in HD females.

Toxicokinetics- Data are summarized in the table below taken directly from the submission (vol 1.5, p 1422). Drug exposures (Cmax, AUC) increased with increasing dose but not dose-proportionally.
Drug concentrations increased with multiple dosing increasing mildly in males and 1.5 to 2-fold in females.

Dosage (mg/kg)	Day of dosing	Sex	C_{max} (ng/mL)	t_{max} (h)	$AUC_{(1-24h)}$ (ng.h/mL)
10	0	M	1340	2	12600
	27	M	1640	1	13280
	0	F	1390	2	14830
	27	F	2080	4	25010
60	0	M	2880	8	35350
	27	M	3590	8	54120
	0	F	2410	8	40910
	27	F	4700	2-4	74320
400	0	M	4690	8	72290
	27	M	5690	8	83910
	0	F	5100	12	106300
	27	F	8550	4	159600

Histopathology (tissues examined from control and HD only)
Macroscopic observations - Thymic red foci in 1 LD, 1 MD, and 2 HD males.
Adrenal- cortical mononuclear cell infiltration in 2/12 C, 5/12 HD females
Lungs-perivascular eosinophilic inflammation-0 C, 1/12 HD M, 2/12 HD F
Spleen-pigmented macrophages in 4/12 HD females
Thymus - moderate localized periarteritis 1/12 HD F
Summary and Conclusions- IC351 [redacted] was well tolerated at all doses and the sponsor defined the NOAEL as 400 mg/kg/day. There were numerous mild effects on hematology and clinical chemistry but no drug-related tissue pathology.
I am concerned that the dose levels studied have not adequately identified target organ toxicity. The top dose was selected based on saturation of absorption above 400 mg/kg. However, the data from the dose-finding study suggest doubling of exposures(AUC) to parent compound and little toxicity with a 2000 mg/kg/day dose (see page 11). In addition, the metabolism studies have revealed the presence of a major metabolite which was not quantified. Parent compound concentrations are not reflective of total compound exposure as evidence by significantly higher exposure to radioactivity than parent drug in drug metabolism studies. The issue of adequacy of dose is complicated by the fact that the in-life portion of the 6 month rat oral toxicity study has already been completed with the same dose levels (10, 60, 400 mg/kg/day).

Dose-Finding Study in the Beagle Dog (study D20786)

The study was conducted according to GLP by [redacted]

Beagle dogs(n = 2/sex) were administered IC351 in 0.5% HPMC with 1%

Tween 80 by gavage as follows:
50 mg/kg/day days 0-1, 100 mg/kg/day on days 2-3; 200 mg/kg/day on days 4-6; 400 mg/kg/day on days 7-8; and 800 mg/kg/day on days 9-10. On days 11-24 dogs were administered 200 mg/kg/day.

Mortality- none

Clinical signs- Sponsors narrative summary describes loose feces (100 mg/kg), pale feces (800 mg/kg), vomiting (200 mg/kg), subdued behavior (200 mg/kg) and thin appearance (1 M, 1F days 12 and 14). Data were not provided so number of animals affected and incidence of observations is unknown.

Body weight- Male dogs lost weight when dosed with 800 mg/kg. Female dogs lost weight when dosed with ≥ 400 mg/kg. Animals regained the lost weight by the end of the study while being dosed with 200 mg/kg/day. Due to thin appearance on days 12 and 14, the pelleted diet was supplemented with pelleted diet mixed with water (mashed food) from days 12-24.

Hematology/Clinical chemistry- not analyzed.

Toxicokinetics- Data are summarized in the table below taken directly from the submission (vol 1.6, pg 1846).

Dosage (mg/kg)	Day of dosing	Sex	Mean C_{max} (ng/mL)	t_{max} range (h)	AUC_{0-24h} (ng.h/mL)
50	0	M	2640	2	21320
		F	2020	4-8	30800
100	2	M	2470	2	39530
		F	4610	2-24	78180
200	4	M	5100	8	79250
		F	6310	8	109100
400	7	M	4020	6-24	75770
		F	4730	2-24	100900
800	9	M	4350	2-8	75830
		F	3830	2-8	49060 †
200	24	M	2690	2-6	32790 †
		F	3630	2-6	45390 †

Cmax : Maximum plasma concentration
Tmax : Time taken to reach Cmax (hours)
AUC 0-24h : Total systemic exposure (Area under the curve)
M/F : Male / Female
† : Contains AUC 0-8h data

Gross pathology - decreased thymic size in 3/4 dogs.
Histopathology-
Lungs- alveolar macrophage infiltration ½ F

Thymus- atrophy 3/4 dogs (1 M, 2 F)
Stomach- inflammatory cell infiltrate 1 M, 1 F
Liver- moderate centrilobular glycogen vacuolization all 4 dogs
Inflammatory cell foci 2 M, 1 F.

Summary and Conclusions- Administration of IC351 was well tolerated up to doses of 400 mg/kg/day. Body weight loss was observed with the 800 mg/kg/day dose in males and females. Thymic atrophy was observed in 3/4 dogs. The sponsor concluded the maximum repeatable daily oral dose in dogs should be 200 mg/kg/day since systemic exposures to parent compound did not increase at higher dose levels.

29-Day Oral Toxicity Study in the Beagle Dog (study D20863)
(lots C2856/116/1 and C2856/121/1)
The study was conducted according to GLP by [redacted] Numerous inconsistencies were noted in the data when audited but are correctly represented in this review.

Beagle dogs (n= 3/sex/dose) were administered 0, 10, 45 or 200 mg/kg/day IC351 in 0.5% HPMC/1% Tween 80 orally by gavage for 29 days. Two additional dogs/sex were treated with vehicle or 200 mg/kg/day for 29 days and maintained untreated for 22 days to study the reversibility of any drug-related effects.

Mortality- One HD male (0004) was killed in extremis on day 14 due to ill health secondary to arteritis. Dosing was stopped on day 11 due to deteriorating condition. Elevations of several white cell parameters including neutrophils, total leukocyte count and monocytes and increased fibrinogen concentration were observed in this dog.

Clinical signs- **Percent Occurrence (out of total observations)**

Observation	Controls	10 mkd	45 mkd	200 mkd	Recovery
Thin appearance	3.4 % M 0% F	5.6% M 0% F	2.2% M 5.6% F	34.3% M 3.4% F	7.1% M 0% F
Loose feces	0% M, F	6.5% M	0% M	5.2% M 13.3% F	28.6% M 9.5% F
Subdued behavior	0% M, F	0% M	3.3% M	3.0% M	
Vomiting	0% M, F	0% M, F	0% M, F	1.5% M 3.3% F	

Body weight- High dose animals of both sexes lost weight in the first week of the study (0.6 kg). The mean body weight of HD males remained less than other groups for the remainder of the study (decreased 16% by 21 days). HD females had mean body wts comparable to other groups on study days 14, 21 and 28.

Food consumption- Group average food consumption was decreased in high dose animals for the first 2 weeks in HD males and the first week in HD females.

Ophthalmology - Report states no drug-related abnormalities. Data were not provided.

Electrocardiography - Recordings were taken 2 to 4 hours after dosing on study days -14 and 24. The Cmax in dogs was 2 hours for the 10 mg/kg dose but 2-12 hours for 45 mkd and 6-15 hours for the 200 mkd dose. Therefore, for the 2 higher dose groups recordings were not coincident with maximum plasma concentrations. IC351 reduced heart rate in high dose dogs of both sexes (Mean initial HR = 160 bpm; decreased 10-15 bpm in C, LD, MD, 40-50 bpm in HD). ST segment elevation, which has been associated with myocardial infarction, pericarditis, and myocardial hypoxia, was observed in 2 HD females (# 24, 32) and may be related to treatment. Other ECG changes of variable P wave amplitude and deep Q and/or S waves were observed at a comparable frequency in control and treated dogs and were considered unlikely to be drug-related.

Hearing Test- Brainstem Auditory Evoked Response was unaffected by treatment with IC351.

Hematology (days 7, 22, 43R)-
HCT/ RBC- Mild elevations (4-10%) in hematocrit and erythrocyte counts were observed in HD females on days 7, 21.
Monocytes- Moderate (70%) increases in monocyte and large stained cell counts were observed in HD males on day 22 and were still present at the end of the 3 week recovery period.
APPT- Slight decreases (3%) in APPT were observed in HD dogs of both sexes on days 7 and 22.

Parameter (units)	Percentage change from control					
	Males			Females		
	7	22	43R	7	22	43R
Haematocrit (L/L)	↓3	0	-	↑9	↑7*	-
Erythrocyte Count (10E12/L)	↓4	↓2	-	↑10*	↑4	-
Monocytes (10E9/L)	↓14	↑74*	↑27	↑1	↑10	↓19
Large Unstained Cells (10E9/L)	↑28	↑72*	↑129	↓12	↑18	↓53
Activated Partial Thromboplastin Time (sec)	0	↓3*	-	↓3	↓3	-

\- Not a protocol requirement
* Statistically significantly different from control (5% level)
↑↓ Indicates a elevation or reduction in that measurement respectively
R Recovery period

Clinical chemistry (days 7,22, 43R)- Changes are summarized in the table below taken directly from the submission (vol 1.6, p 1934).
Alk P, ALT, AST- Decreases were observed at all sampling times in HD males and on days 7 and 22 in HD females.
Bilirubin- significantly decreased in HD dogs of both sexes on day 22
Cholesterol- increased in HD dogs on days 7, 22
Changes in bilirubin and cholesterol were reversible by day 43.

Parameter (units)	Percentage change from control					
	Males			Females		
	7	22	43R	7	22	43R
Alkaline phosphatase	↓19*	↓19*	↓9	↓18**	↓2	↑20
Alanine aminotransferase	↓44**	↓66**	↓39	↓19**	↓22	↓24
Aspartate aminotransferase	↓39**	↓35**	↓9	↓32*	↓23	↑12
Bilirubin	↑3	↓26**	0	↓7	↓20*	↑25
Cholesterol	↑43**	↑28*	↓12	↑29*	↑14	↓1
Glucose	↓8*	↓5	-	↓1	↓4	-
Potassium	↑1	0	-	↑10*	↑3	-

\- Not a protocol requirement
* Statistically significantly different from control (5% level)
** Statistically significantly different from control (1% level)
↑↓ Indicates a elevation or reduction in that measurement respectively
R Recovery period

Urinalysis- specific gravity of the urine was the only parameter measured and was significantly decreased in MD, HD female groups.

Organ weights -No treatment related effects on organ weights.

Toxicokinetics-

Dosage (mg/kg)*	Day of dosing	Sex (M/F)	C_{max} (ng/mL)	t_{max} (h)	$AUC_{(0-24h)}$ (ng.h/mL)
10	0	M	764	2	7760
	28	M	682	2-6	8060
	0	F	865	2	4300
	28	F	438	2-3	3280
45	0	M	2940	2-12	39200
	28	M	1040	2	7090
	0	F	1570	2-3	12800
	28	F	1910	6-9	24300
200	0	M	4440	6-9	67100
	28	M	11500	9-15	220000
	0	F	5680	9-12	70600
	28	F	7200	9	135000

Histopathology-(n = 3/sex/dose pathologic evaluation was not performed on recovery animals). Disseminated arteritis was observed in the brain, spinal cord, lungs, and thymus of the high dose male killed in extremis on study day 14. Perivascular inflammation in the lungs was observed with increased frequency in IC351-treated dogs.

Tissue/Finding	0 mg/kg/d	10 mkd	45 mkd	200 mkd
Brain-endothelial swelling, leucocyte pavementing, meningeal inflammation				1/3 M
Gall bladder, lymphoid foci	2/6	4/6	5/6	5/6
Heart, coronary periarteritis	1/6			
Liver, hepatocellular vacuolization			1/3 F	1/3 F
Lung, perivascular inflammation periarteritis, bronchial a.		3/6,1M/2F	3/6,1M/2F	2/6HD, 1M, 1F 1/3 M
Spinal cord, periarteritis				1/3 M
Thymus, periarteritis				1/3 M

A supplementary examination of the hearts from all animals killed on study day 29 revealed changes in treated animals which had not been observed in the standard sections examined in the initial study. Arteritis of the right coronary artery was seen in 1 MD and 1 HD dog. No coronary arteritis was observed in the one HD dog that was killed day 14 that had arteritis at other non-coronary sites. No additional cases of coronary arteritis were observed in controls (other than 1 case previously identified above) or in dogs dosed with 10 mg/kg/day. A supplemental evaluation of the coronary vasculature revealed an increased incidence of coronary arterial lesions in mid and high dose dogs(see table below). These coronary lesions were not associated with vasodilation/tachycardia as has been previously described. No changes in heart rate were observed in mid dose dogs and mild to moderate decreases in heart rate were observed in high dose dogs.

	21F control Killed at term	3M inter Killed at term	19F inter Killed at term	4M high Sac killed D14	32F high Killed at term
clinical signs	silent	silent	silent	present	silent
macroscopic changes	cardiac nodule	none	none	none	none
other organ involvement	no	no	no	arteritis	no
coronary involvement	arteritis	intimal thickening	arteritis	none	arteritis

Shaded animals are those in which arterial lesions had been seen in the initial study

Arteritis in Six Month Dog Study

4 HD dogs died or were killed in extremis as follows - #8M day 35, #24M day 71, #28F day 151, # 46F day 93.

Controls	400 mkd
4/8 M - minimal/moderate 3 coronary artery, 1 bladder	5/8 M moderate/severe disseminated #4 - heart, moderate #8- brain, heart, esophagus, thymus, severe #12- thymus, moderate #24- heart, thymus, bladder, severe #20R -heart, thymus, spinal cord, moderate
1/8 F- minimal coronary artery	3/8F- moderate/severe disseminated #28- thymus, spinal cord, moderate #46- brain, heart, lungs, ovaries, stomach, thymus, mammary gland, bladder, mod/severe #40R- thymus, mild

The study pathologist, [redacted]. concluded "The high incidence of arteritis that has been associated with high doses of (IC351)in the 6 month dog study, and the predominance of arterial changes in the high and intermediate dose groups in the one month study are strongly suggestive of a treatment related change or treatment related exaccerbation of the spontaneous polyarteritis".

Conclusions- One high dose dog was replaced on day 2 and one HD dog was killed in extremis on day 14 due to deteriorating clinical condition. The dog killed on day 14 was observed to have moderate to marked arteritis in the brain, lungs, spinal cord and thymus. Drug treatment increased erythrocyte counts in HD females and monocyte/large unstained cell counts in HD male dogs. Decreases in alkaline phosphatase, ALT, AST, and bilirubin, and increases in cholesterol were observed in high dose dogs of both sexes. Drug-related changes in organ weights or histopathology were not observed. The dose-related pattern of coronary arteritis and disseminated arteritis in the one and 6 month studies strongly suggest it is drug-related. The sponsor concludes it is drug-related exacerrbation of beagle pain syndrome. Spontaneous polyarteritis or beagle pain syndrome is described with a rare occurrence in some colonies. The description of disseminated arteritis in 1/6 HD dogs in the one month study and 4/12 dogs in the 6 month toxicity study with IC 351 suggests the finding is drug-related. In addition, drug-related vasculitis was observed in mid- and high dose dogs in the one, six and twelve month oral toxicity studies for the PDE 5 inhibitor Viagra.

GENOTOXICITY

Microbial Mutagenicity Study with IC351 (study #U20206; report WPT/93/607)

The study was conducted according to GLP by [redacted]

[redacted] (IC351) was tested in the Ames and [redacted] assays in tester strains Salmonella typhimurium TA1535, 1537, 100, 98, and E.coli WP2 [redacted] and WP2uvrA [redacted]. Concentrations of 15, 50, 150, 500, 1500, and 2500ug/plate [redacted] in DMSO with and without metabolic activation (S9) were tested in replicate assays (2 Ames and [redacted] assays). [redacted] produced no cytotoxicity but significant precipitate was observed at doses > 1500 ug/plate so 5000 ug/plate was not tested in the definitive mutagenicity assays. [redacted] showed no evidence of mutagenic activity in the Ames tests or [redacted] assays in the presence and absence of metabolic-activation.

Conclusion- [redacted] was not mutagenic in any of the bacterial strains tested in the Ames and [redacted] assays.

Mouse Lymphoma Thymidine Kinase Mammalian Cell Mutation Test (study V21166)

The study was conducted according to GLP by [redacted] in June, 1996.

L5178 cells were exposed to [redacted] at concentrations of 10, 25, 50 and 75 ug/ml ± S9 for 3 hours. Cells were plated with trifluorothymidine for 2 days after treatment. After an additional 11 days, plates were scored for the presence of colonies resistant to the lethal effects of trifluorothymidine. Mutant frequency was not affected by treatment of L5178 cells with 10-75 ug/ml [redacted] Only a summary table of the data was provided. Relative survival was not significantly affected by the concentrations tested (0-14% reduction in cell survival at the highest dose tested). ICH and OECD protocols require selection of doses up to those producing 80% reduction in relative survival.

Conclusion- The study needs to be repeated using higher dose levels. In addition, the complete data set should be provided for review.

In Vitro Cytogenetic Evaluation of IC 351 in Cultured Human Lymphocytes (study V20918, report WPT/95/293)

The study was conducted from March-June 1995 according to GLP at [redacted]

[redacted] was evaluated for clastogenic effects in cultured human lymphocytes. Four tests were analysed for chromosome aberrations as follows:

Without metabolic activation:

1. 5,10,20,40,60,80,100,125 and 150 ug/ml; 20.5 hr harvest
2. 10, 20, 40 ug/ml [redacted] treated for 20.5 hours with harvest times at 20.5 and 44.5 hours.

With metabolic activation (rat liver S9):

1. 1,5,10,20,and 40 ug/ml treated for 3 hours, harvested after a further 19.5 hrs (22.5 hours from beginning of treatment. Metaphase analyses were performed on the lowest 3 doses only since precipitation in the cultures was observed at doses ≥ 10 ug/ml.
2. 1, 5, and 10 ug/ml treated for 3.5 hours harvested at 46.5 hours

Dose levels were limited by solubility. The highest doses without S9 produced 20-30% reductions in the mitotic index. The highest dose with S9 produced 4-14% reductions in the mitotic index. [redacted] did not induce chromosome aberrations in the absence of S9 mix at either harvest time (20.5 or 44.5 hours). Similarly, [redacted] did not increase chromosome aberrations in the presence of S9.

Conclusion- IC351 was not clastogenic in cultured human lymphocytes in the absence or presence of S9 mix.

WHO Nitrosation Assay with [redacted] (report WPT/95/232)

The study was conducted by [redacted]
The test determines nitrosatability of indolic drugs under acidic conditions.

10 mM IC351 and 40 mM sodium nitrite were incubated under acidic conditions for 1 and 4 hours. The reaction mixtures were neutralized and assayed in the standard Ames test (plate incorporation) for direct acting mutagens and the [redacted] test for indirect acting mutagens. Negative results were obtained in the nitrosation assay procedure in the absence and presence of S9 mix.

Conclusion- [redacted] was not nitrosated in vitro to form mutagenic products.

Summary and Conclusions-

IC 351 [redacted] is a competitive inhibitor of cGMP-phosphodiesterase type 5 and is proposed for the treatment of male erectile dysfunction. IC_{50} values for some PDE isozymes are:
40uM for PDE3A (cardiac myocytes, vascular smooth muscle, platelets)
20uM for PDE 3B (adipocytes and leukocytes),
3.4 uM for PDE 6 (retina), and 0.001-0.0025 uM for human recombinant PDE5. Based on these data, IC 351 has a 10,000-fold selectivity for PDE type 5 over PDE type 3. In cultured cells (vascular smooth muscle, renal epithelium, umbilical vein epithelium), IC 351 increases basal levels of cGMP approximately 4-fold and potentiates cGMP levels produced by guanylyl cyclase activating agents such as ANF or sodium nitroprusside (4-200 fold).

IC 351(1-5 mg/kg,po) produces significant, long-lasting reductions in blood pressure in hypertensive rat models. IC 351 has only modest effects on blood pressure in normotensive rats. Similarly, oral doses of IC351 from 3 to 200 mg/kg to beagle dogs produced no change in blood pressure in some animals or mild(10%) changes in blood pressure and heart rate. IC 351(0.3 mg/kg,iv) produced significant diuresis and increased sodium excretion in rats. Data regarding drug effects on GI motility were not provided.

Acute toxicity studies demonstrated the oral lethal dose was > 2000 mg/kg in both rats and mice. The intravenous lethal dose was approximately 100 mg/kg in mice and > 62.5 mg/kg in rats. Significant signs of toxicity (subdued behavior, labored/rapid breathing, prostration, tremor and convulsions) were observed in rats and mice treated with single intravenous doses ≥37.5 mg/kg.

One month oral toxicity studies were conducted in Wistar rats and beagle dogs. **Rats** were administered doses of 10, 60 and 400 mg IC 351/kg/day. There was no drug-related mortality or effects on food consumption or body weight. Reticulocyte counts and thrombotest clotting time increased in all drug treated groups. White blood cells, leukocyte counts, lymphocytes, and monocytes increased in mid and high dose females. The hematology changes were reversible after a 3 week recovery period. Clinical chemistry changes included decreases in bilirubin, total protein, and albumin in mid and high dose male and female rats. Male rats also displayed decreased alkaline phosphatase and cholesterol, and increased BUN at all dose levels. Changes in clinical chemistry were also reversible. Pathologic observations included hemorrhagic foci in the thymus (1 LD, 1 MD, 2 HD males) and moderate periarteritis in the thymus of one HD female. Perivascular eosinophilic inflammation was observed in the lungs of three high dose rats (1 M, 2 F). Pigmented macrophages were observed with increased frequency in the spleen of high dose female rats (0 controls, 4/12 HD). Histopathologic evaluation of tissues from the low and mid dose groups were not conducted. A NOEL was not identified.

Dogs were administered doses of 10, 45 and 200 mg IC 351/kg/day. One high dose dog was killed moribund on day 14 due to ill health secondary to marked, disseminated arteritis. High dose animals had decreased food consumption and lost weight the first week of the study. Females regained the lost weight by day 14, males continued to have decreased gain relative to other groups throughout the study. The highest dose of 200 mg/kg produced a moderate decrease in heart rate in HD dogs of both sexes. Two high dose females also displayed ST segment elevations (indicative of myocardial infarction, pericarditis, myocardial hypoxia). High dose female dogs had mild elevations in hematocrit and erythrocyte counts; high dose males had marked increases in monocyte and large stained cell (?) counts which were still present after a 3 week recovery. High dose dogs of both sexes had decreases in alkaline phosphatase, ALT, AST, and bilirubin. Increased serum cholesterol was also observed in HD dogs of both sexes.

Histological evaluation revealed marked disseminated arteritis in the dog killed in extremis and an increased frequency of perivascular inflammation in the lungs of dogs in all treated groups. In addition, a supplemental evaluation of the coronary vasculature suggests an increased incidence of coronary arterial lesions in mid and high dose dogs. A preliminary report from the 6 month dog study was also provided which described that four high dose dogs were killed due to multiple (>5) episodes of beagle pain syndrome (spontaneous polyarteritis). A total of 7 dogs (1 MD, 6 HD) displayed clinical signs of polyarteritis during the study. Histopathologic evaluations in the 6 month study revealed minimal arteritis of the coronary artery of some control dogs and no arteritis in dogs treated with the lowest dose of 10 mg/kg/day. The mid and high dose dogs displaying clinical signs had pathologic evidence of moderate to marked disseminated arteritis in multiple organs (see page 21). The sponsor claims drug/stress-induced exacerbation of spontaneous polyarteritis. I have reviewed numerous dog studies conducted by [redacted] and have never seen "beagle pain syndrome" reported previously despite use of high doses which stress dogs. ([redacted] consistently pushes the high dose to identify target organ toxicity).

Drug-induced arteritis has been reported for other PDE type 3, type 4, and type 5 inhibitors in multiple species including mice, rats and dogs. [redacted] has halted development of their type 3 inhibitors due to the vasculitis findings. Other PDE type 4 inhibitors have been placed on clinical hold in the Div of Pulmonary Drug Products due to the absence of a safety margin between doses which produce vasculitis in animals and human therapeutic exposures. Pfizer observed drug-induced vasculitis in rats and dogs treated with sildenafil (Viagra). Pfizer concluded that although the pathologic findings in dogs were consistent with those observed in spontaneous polyarteritis, the frequency was far in excess of the normally rare occurrence of beagle pain syndrome and, therefore, must be concluded to be drug-related.

The available single dose human pharmacokinetics data from young, healthy volunteers suggest the exposure multiples for the 200 and 400 mg/kg/day doses in dogs are less than 10 times human exposures. Therefore, it was recommended that the reports from the completed 6 month rat and dog toxicology studies and the PK data from the ongoing multidose (7 days) pharmacokinetics study in elderly men be submitted for review. This data should permit us to better define a safe dose for use in man. We are concerned because the proposed trial is a 3 week Phase II study in 300 men with erectile dysfunction. The plan is aggressive since a safety margin can not be established from the preclinical data provided and the drug has not been previously administered to elderly men with vascular disease. (It has been demonstrated with other PDE inhibitors and agents that produce vasculitis that older animals or animals with existing vascular disease are more sensitive to the drug-induced vasculitis).

Recommendations

The IND was placed on clinical hold and numerous recommendations were communicated to the sponsor by telecon and in a clinical hold letter. The original safety review, minutes of the telecon placing the IND on hold, and clinical hold letter are appended.

Additional comments to be communicated to the sponsor are:

1. Please conduct safety pharmacology studies to assess the effects of IC 351 on gastrointestinal motility and gastric acid secretion.

2. The Mouse Lymphoma Mammalian Cell Mutation Assay should be repeated using higher dose levels which produce significant cytotoxicity consistent with the requirements defined in OECD and ICH guidelines. In addition, the complete data set should be provided for the assay submitted n the original IND [redacted]

3. The results of an in vivo genotoxicity assay should also be provided prior to Phase II clinical trials.

Jeri El-Hage 5/28/98

Jeri El-Hage, Ph.D.

cc: IND 54,553, HFD-580 IND
HFD-580/A Jordan/T Rumble/J El-Hage
54553.#1

IND 54,554
IC 351

APPENDIX I

SUMMARY TABLES FOR ADME STUDIES

Table 4.2

IC351 Forms and Formulations Used *In Vivo* for Absorption, Distribution, Metabolism and Excretion (ADME) Studies

Study No	Report No	Study Title	IC351 Dose Formulation
R21147	[redacted]	Pilot Study to Assess the Absorption Profile of [redacted] (IC351) Coprecipitate Following Oral Dosing in the Han Wistar Rat	[redacted]
D21148		[redacted] (IC351): Study to Assess the Absorption Profile in the Beagle Dog	
BPW662		The Pharmacokinetic Profile of [redacted] (IC351) in the Han Wistar Rat Following a Single Intravenous or Single Oral Dose of ^{14}C- [redacted] (10 mg/kg)	
BPW549 BPW564		Identification of Metabolites of [redacted] (IC351) Excreted in Rat and Dog Bile (3 mg/kg *iv*)	
BPW618		The Distribution, Metabolism, and Excretion of [redacted] (IC351) in the Han Wistar Rat after a Single Intravenous or Single Oral Dose of ^{14}C- [redacted] (10 mg/kg)	
BPW641		The Pharmacokinetics, Metabolism, and Excretion of [redacted] (IC351) in the Beagle Dog after a Single Oral Dose of ^{14}C- [redacted] (10 mg/kg)	
BPW659	[redacted]	The Pharmacokinetics, Metabolism, and Excretion of [redacted] (IC351) in the Beagle Dog after a Single Intravenous Dose of ^{14}C- [redacted] (10 mg/kg)	[redacted]

Table 4.3

Pharmacokinetic Parameters for Orally Administered IC351 in the Rat (Part 1)

Report No. (Study No.)	Duration of Dosing		Dose Level (mg/kg)	$AUC_{0\rightarrow24}$ mean[1] (ng. h/mL)	
				M	F
[redacted] (BPW662)	1		10	9,591	12,335
[redacted] (R21147)	Maximum Repeatable Dose Study		50	37,400	45,800
			100	66,000	70,900
			200	77,000	93,600
			400	145,000	152,000
			800	140,000	248,000
			1200	140,000	NC[2]
[redacted] (R20861)	4 Weeks	Day 0	10	12,600	14,830
			60	35,350	40,910
			400	72,290	106,300
		Day 27	10	13,280	25,010
			60	54,120	74,320
			400	83,910	159,600
[redacted] (R21236)	26 Weeks	Day 0	10	5,600	14,700
			60	34,500	46,000
			400	92,600	121,000
		Day 25	10	7,890	18,800
			60	38,800	47,000
			400	53,800	122,000
		Day 77	10	12,200	24,300
			60	36,500	79,900
			400	61,600	130,000
		Day 168	10	14,900	28,200
			60	29,100	82,900
			400	72,200	190,000

[1] IC351 levels determined from plasma samples.
[2] NC: not calculated due to absence of data for 24 hours after dosing.

Table 4.3

Pharmacokinetic Parameters for Oral Administered IC351 in the Rat (Part 2)

Report No. (Study No.)	Duration of Dosing		Dose Level (mg/kg)	T_{max} (h)		C_{max} mean (ng/mL)[1]	
				M	F	M	F
[redacted] (BPW662)	1		10	1	2	788	856
[redacted] (R21147)	Maximum Repeatable Dose Study		50	4	4	3,210	3,670
			100	4	4	5,160	5,680
			200	4	8	5,190	5,760
			400	4	2	8,370	9,910
			800	8	4	8,500	11,600
			1,200	4	6	7,830	12,100
[redacted] (R20861)	28 Days	Day 0	10	2	2	1,340	1,390
			60	8	8	2,880	2,410
			400	8	12	4,690	5,100
		Day 27	10	1	4	1,640	2,080
			60	8	2-4	3,590	4,700
			400	8	4	5,690	8,550
No Report (R21236)	26 Weeks	Day 0	10	2	2	758	1,290
			60	2	4	2,245	3,590
			400	8	8	6,040	8,355
		Day 25	10	1	2	982	1,600
			60	2	4	2,260	3,605
			400	8	8	4,305	7,090
		Day 77	10	2	2	1,345	1,745
			60	4	8	3,230	5,900
			400	4	2	5,155	8,070
		Day 168	10	2	2	1,425	2,455
			60	4	2	2,105	5,760
			400	4	2	5,350	10,470

[1] IC351 levels determined from plasma samples.

[redacted]

Table 4.4

Pharmacokinetic Parameters for Oral Administered IC351 in the Beagle Dog (Part 1)

Report No. (Study No.)	Duration of Dosing		Dose Level (mg/kg)	$AUC_{0\rightarrow24}$ Range (ng. h/mL)[1]		$AUC_{0\rightarrow24}$ mean[1] (ng. h/mL)	
				M	F	M	F
[redacted] (BPW641)	1		10	[redacted]		4,109	2,278
[redacted] (D20786)	Maximum Repeatable Dose Study		50			21,320	30,800
			100			39,530	78,180
			200			79,250	109,100
			400			75,770	100,900
			800			75,830	NC[2]
D21148 (D21148)	Maximum Repeatable Dose Study (10 Days)		50			43,600	64,500
			100			76,800	11,500
			200			90,200	157,000
			400			134,000	175,000
			600			132,000	204,000
[redacted] (D20863)	4 Weeks	Day 0	10			7,760	4,300
			45			39,200	12,800
			200			67,100	70,600
		Day 28	10			8,060	3,280
			45			7,090	24,300
			200			220,000	135,000
No Report (D21235)	26 Weeks	Day 1	10			12,835	10,205
			60			8,300	51,100
			400			115,500	109,000
		Day 35	10			21,230	10,335
			60			11,700	68,700
			400			144,500	158,500
		Day 77	10			12,732	8,015
			60			56,055	31,943
			400			134,017	109,338
		Day 182	10			19,027	14,762
			60			72,525	49,650
			400			117,567	127,833

[1] IC351 levels determined from plasma samples.
[2] NC: not calculated due to absence of data for 24 hours after dosing.

Table 4.4

Pharmacokinetic Parameters for Oral Administered IC351 in the Beagle Dog (Part 2)

Report No. (Study No.)	Duration of Dosing		Dose Level (mg/kg)	T_{max} (h)		C_{max} Range[1] (ng/mL)		C_{max} mean[1] (ng/mL)	
				M	F	M	F	M	F
[redacted] (BPW641)			10	1-3	1-72	[redacted]		526	416
[redacted] (D20786)	Maximum Repeatable Dose Study		50	2	4-8			2,640	2,020
			100	2	2-24			2,470	4,610
			200	8	8			5,100	6,310
			400	6-24	2-24			4,020	4,730
			800	2-8	2-8			4,350	3,830
D21148 (D21148)	Maximum Repeatable Dose Study (10 Days)		50	2-8	8-24			2,720	4,040
			100	1-2	2-8			4,220	6,160
			200	2-4	2-8			4,800	7,410
			400	4	2-8			7,850	8,400
			600	4-6	2-4			7,530	11,100
[redacted] (D20863)	29 Days	Day 0	10	2	2			764	865
			45	2-12	2-3			2,940	1,570
			200	6-9	9-12			4,400	5,680
		Day 28	10	2-6	2-3			682	438
			45	2	6-9			1,040	1,910
			200	9-15	9			11,500	7,200
No Report (D21235)	26 Weeks	Day 1	10	2-9	1-12			1,064	813
			60	3	12-15			1,135	3,460
			400	12	9			6,520	5,765
		Day 35	10	2-9	2			1,355	1,040
			60	2-3	2-12			1,155	3,985
			400	2-9	9			7,820	7,735
		Day 77	10	2-6	1-4			938	986
			60	2-15	1-9			3,302	2,365
			400	2-12	3-4			7,093	7,401
		Day 182	10	2-9	1-12			1,415	1,209
			60	2-9	2-15			4,235	3,218
			400	2-9	2-12			6,948	7,288

[1] IC351 levels determined from plasma samples.

Table 4.5

A Comparison of Pharmacokinetic Parameters for Intravenous IC351

Report No. (Study No.)	Species	Route of Administration	Duration of Dosing	Dose Level (mg/kg)	$AUC_{0\rightarrow24}$ Range (ng. h/mL)		$AUC_{0\rightarrow24}$ mean (ng. h/mL)	
					M	F	M	F
[redacted] (BPW662)	Rat	Intravenous	1	10	[redacted]		18,064	36,659
[redacted] (BPW641)	Dog	Intravenous	1	10			23,532	24,705

Report No. (Study No.)	Species	Route of Administration	Duration of Dosing	Dose Level (mg/kg)	C_{max} Range (ng/mL)		C_{max} mean (ng/mL)	
					M	F	M	M
[redacted] (BPW662)	Rat	Intravenous	1	10	[redacted]		3,700	5,670
[redacted] (BPW641)	Dog	Intravenous	1	10			3,663	4,487

IND 54,553

8 380

[redacted]

Table 1. Completed or Ongoing Studies - Clinical Pharmacology

Study ID	Study	Sites-status	Objective	Design	Number	Entry Criteria	Endpoints	Actions
C95-031	First human dose	1 site UK completed	Safety Toleration PK	4 way cross-over 1-500 mg	17	Healthy male subjects	PK Safety Toleration	Multiple dose selection
C95-050	Pilot bioequiv-alence and food effect	1 site UK completed	New CT Food effect	3 way cross-over 100 mg	13	Healthy male subjects	PK Safety	Phase 2 dosing instructions
C95-064	Mulitple dose PK	1 site Germany completed	Safety PK	Parallel 8 days dose 50 mg,	27	Healthy male subjects	PK Safety Toleration	Phase 2 dose selection
DSD02	Multiple dose PK Elderly	1 site Netherlands completed	Safety PK	Parallel 7 days 10, 50,100 mg	34	Healthy elderly male subjects	PK Safety Toleration	Increase age range in Phase 2
LVAA	ADME	1 site US ongoing	ADME profile	100mg ^{14}C 100uCi	6	Healthy male subjects	Metabolic fate	Biopharm study design
LVAB	Nitrate interaction	1 site US ongoing	Safety	10 mg And Viagra	22	Healthy male subjects	Effect of co-admin with Nitrates	Phase 3 entry criteria and label

Abbreviations: PK = pharmacokinetic, ADME = Absorption, Distribution, Metabolism, Excretion

Table 2. Completed or Ongoing Studies - Phase 2 Efficacy and Safety

Study ID	Study	Sites-status	Objective	Design	Number	Entry Criteria	Endpoints	Actions
DSD01	PD	3 sites Europe completed	PD Safety PK	2 way cross-over DB placebo SD 100 mg	44	Mild to moderate MED	PD safety	Proof of concept
DSD04	Dose ranging efficacy in MED with daily dosing	19 sites Europe completed	Efficacy Safety Population PK	DB parallel placebo, 21 day dosing, 10, 25, 50, 100 mg	294	Mild to moderate MED No angina No nitrate therapy	Efficacy Q3 and Q4 IIEF safety	Phase 3 dose and regimen selection
DSD06	Dose ranging efficacy in MED with on demand dosing	13 sites US completed	Efficacy safety Population PK	DB parallel placebo, 14 doses in 21 days, 2, 5, 10, 25 mg	179	Mild to moderate MED No angina No nitrate therapy	Efficacy Q3 and Q4 IIEF safety	Phase 3 dose and regimen selection
LVBE	Open label safety	15 sites Europe	Safety	Open titration 5, 10, 25, 50 mg	200	Mild to moderate MED From DSD04	Safety	Phase 3 dose selection, Submission dossier
LVBD	Open label safety	15 sites Europe	Safety	Open titration 5, 10, 25, 50 mg	200	Patients from all IC351 trials	Safety	Phase 3 dose selection, Submission dossier
LVAC	Dose ranging efficacy in MED with on demand dosing	15 sites in Canada	Efficacy Safety Population PK	DB parallel placebo 2, 5, 10 and 25 mg for 8 weeks	225	Mild to severe MED NYHA stage I No nitrate therapy	Efficacy (IIEF Q3 and Q4) Safety	Phase 3 dose and regimen selection

Abbreviations: DB = double blind, IIEF = International Index of Erectile Function, MED = Male Erectile Dysfunction; PK = pharmacokinetic, PD = pharmacodynamic

IND 54,553
IC 351

Div. of Reproductive and Urologic Drug Products, HFD-580
Reviewer: Jeri El-Hage, Ph.D.

Drug: IC351, Phosphodiesterase Type 5 inhibitor

Sponsor: ICOS Corporation, Bothell, WA 98021

Submission Dates: #002 3-13-98; # 003 4-24-98; #004 , #005 7-3-98

Review of Pharmacology and Toxicology Data
Review # 2

Amendment # 002

This amendment was submitted in response to the clinical hold placed on IND 54,553 in December, 1997. This submission contains a draft report for the six month oral toxicity study in beagle dogs and the conclusions of consultant cardiovascular pathologists, Drs . [redacted] , hired by the company to interpret the results.

The report from Dr. [redacted] is identical to that presented previously in the original IND submssion and simply attributes all findings to BPS. The review by Dr. [redacted] is new and , in my opinion, more scientifically sound. Dr. [redacted] dismisses the atrial lesions described in the one month dog study stating they are observed in a frequency consistent with spontaneous coronary lesions in dogs and are not indicative or diagnostic of drug-induced arteritis. I agree with this conclusion and never felt that the mild coronary artery lesions observed in all dose groups including controls were drug-related. Beagles are known to have a moderate (30-40%) background incidence of mild right coronary artery lesions. I continue to be concerned about the moderate to severe disseminated arteritis observed in high dose dogs in the one and six month toxicity studies which is explained by the sponsor as beagle pain syndrome. The published data on PDE 3 inhibitor vasculitis is limited and does not completely describe the pathology or distribution of the lesions. In fact, numerous publications describe PDE 3 and PDE 4-induced vasculitis has been demonstrated in many tissues in addition to coronary arteries including the spleen, lungs, kidneys, thymus, mesenteric arteries, pancreas, testes, meninges. Additional unpublished data submitted to the agency under various INDs describe PDE 3, PDE 4 and PDE 5 - induced arterits in numerous tissues, consistent with those observed in beagle pain. In addition, the pathologic findings associated with PDE 3 and 4 inhibitor-induced vasculitis are consistent with the pathology described in IC351-treated dogs, namely, medial hemorrhage and necrosis, periarteris, arteritis in arteries and periphlebitis, thrombosis and apoptosis in veins. Inflammatory infiltrate consisting of leukocytes, lymphocytes, and macrophages have been described in all three layers of the arterial wall (intima, media and adventitia). Thrombi consisting of platelets, fibrin, erythrocytes and leukocytes are observed in veins. In addition, the consultants suggest that PDE inhibitor arteritis is only observed at doses which produce marked hemodynamic effects (vasodilation, hypotension, tachycardia, hypoperfusion). There is considerable in-house data demonstrating that PDE 3, PDE 4 and PDE 5 inhibitors produce vasculitis in dogs and rats at doses which have no effect on systemic hemodynamics . In addition, [redacted] has studied regional blood flow in rats in the specific mesenteric arterial beds susceptable to PDE 3/PDE 4 inhibitor induced arteritis, presuming regional flow was affected despite an absence of effects on systemic blood pressure or heart rate. SKB

was unable to demonstrate any changes in regional flow in mesentaric beds contributing to the drug-induced arteritis in rats.

Six Month Oral Toxicity Study with IC 351 in Beagle Dogs ((Study D21235)

The data presented are an unaudited draft report issued by [redacted] The study was performed by [redacted] from March 25 – October 25, 1996 and data tables from the in-life portion of the study were provided by [redacted]

Fasted beagle dogs (n = 6/sex) were administered [redacted] orally by gavage at doses of 0 and 400 mg/kg/day and groups of 4/sex were dosed with 10 and 60 mg/kg/day . Vehicle was a suspension of hydroxypropylmethylcellulosephthalate in sterile water containing 1% Tween 80. The 2 additional animals /sex in the control and HD groups were killed after a 4 week drug-free recovery period.

Mortality - Four high dose dogs were killed moribund due to recurrent (≥ 5) episodes of symptoms consistent with beagle pain syndrome (BPS). The animals were killed as follows: 8M on day 36, 24 M on day 71, 28F on day 151, 46F on day 93.

Signs- Seven dogs, 6 high dose (3M, 3F) and 1 mid dose (M) developed clinical signs during the study. Signs included thin appearance, subdued behavior, stiff neck and pyrexia. Initially the signs in the affected animals (listed in the table above) were considered not drug-related and were treated with antibiotics and corticosteroids. From day 133 animals were no longer treated for symptoms and were allowed to recover from up to 5 episodes of arteritis.

Arteritis Findings in Animals with Clinical Signs

Dose, mg/kg	Animal #	Onset	Day of Death	Tissues with Arteritis
60	15M		TK	spinal cord, thymus
400	8M	d 20	D 36	brain, coronary arteries, esophagus, thymus
400	12M		TK	thymus
400	24M	d 42	D 71	right atrium, mitral valve, thymus, urinary bladder
400	28F	d 46	D 151	thymus
400	40F		TK	thymus
400	46F	d 3	D 93	brain, heart (atrium, RV, AV valve, SA node), lungs, ovaries, mammary gland, stomach, thymus, urinary bladder

Animals 8 , 24 M and 28, 46 F were the most severely affected and were killed in extremis on the day indicated. Other animals were killed at study termination (TK = terminal kill).

Body weight - reduced b.w. gain in mid dose females and high dose animals of both sexes during the first 12 weeks of treatment. Data are summarized in the table below taken directly from the submission (vol. 3.1, p131)

Food consumption-significantly decreased in MD females, HD both sexes during first week of dosing (14% in males ;44% in females). Normal thereafter.

Ophthalmology/Hearing Test- no treatment related findings.

Electrocardiography-
Recordings were made 2-4 hours after dosing while Tmax was 9-12 hours in mid and high dose dogs. Sponsor states there were no drug-related changes in ECG but data were not provided. The test describes the the experimental lab described ST segment deviations in 3/12 C, 2/8 LD, 2/8 MD and 6/12 HD (25%, 25%, 25% and 50% of dogs, respectively), The sponsor hired [redacted], a veterinary cardiologist to read the ECG tracings and he concluded the observations were within normal limits.

Hematology- Polyarteritis has been associated with neutrophilia and raised erythrocyte sedimentation rate. Hematology samples from animals killed during an arteritis epidsode showed elevated neutrophil and fibrinogen levels. However, the group mean values show only modest increases which are summarized in the table below taken directly from the submission (vol 3.1, p 132). Decreases in red blood cells were observed in high dose animals of both sexes. In male dogs
decreases in RBCs and hemoglobin were still observed at the end of the drug-free recovery period.

Blood chemistry-
Alkaline phosphatase(ALK)- were increased in both sexes receiving 60 or 400 mg/kg/day from day 28 onward. Levels were still elevated at the end of the recovery period.
Alanine aminotransferase- lowered in the high dose animals of both sexes throughout the treatment period. Findings were reversed after drug-free interval.
Aspartate aminotransferase- lowered in high dose animals of both sexes on day 28 only.

Blood Chemistry Findings

Two additional findings in this study previously associated with polyarteritis were decreased plasma albumin levels in high dose dogs and reduced plasma calcium levels dogs treated with 60 and 400 mg/kg/day.

Toxicokinetics- Data are summarized in Table A below taken directly from the submission (vol . 3.1, pg 197). Exposures were very variable and , therefore, ranges for PK values were provided rather than mean values. Significant drug accumulation was observed with multiple dosing (2-10 fold). There were no apparent sex differences in metabolism.

Organ weights- Dose-related increases in liver weight were observed in treated males and females. The sponsor considers the liver weight increase to be secondary to increased metabolism as no significant liver pathology was observed. .
Dose-related decreases in testicular weight were observed in treated males and were associated with histopathologic evidence of testicular atrophy in mid and high dose dogs. Decreased testicular weights and atrophy were not reversible after a one month drug-free recovery period.

Relative Organ Weights (g) and Percent Change from Control

Organ	Control	10 mg/kg	60 mg/kg	400 mg/kg
Liver Males	392	374	404	469 (↑ 20%)
Females	360	425 (↑ 18%)	443 (↑ 23%)	448 (↑24%)
Testes	29.5	24.4 (↓ 17%)	17.7 (↓ 40%)	17.4 (↓ 41%)

IND 54,553
IC 351, LY 450190

Jordan

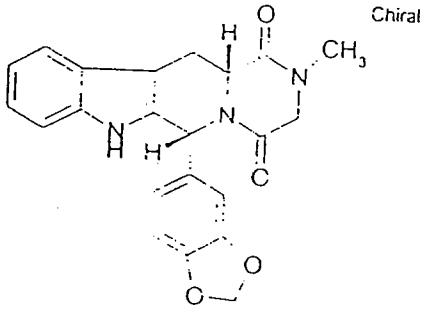
AUG 10 1999

REVIEW AND EVALUATION OF PHARMACOLOGY/TOXICOLOGY DATA:

KEY WORDS: Erectile Dysfunction, Phosphodiesterase inhibitor, IC351, LY450190
Reviewer Name: Jeri El-Hage, Ph.D.
Division Name: Reproductive and Urologic Drug Products
HFD#: 580
Review Completion Date: June 14, 1999
Review number: 3
IND/NDA number: 54,553
Serial number/date/type of submission: #017, IT, 1-14-99 (1 volume)
#022, IT , 3-30-99 (5 volumes)
#026, 5-10-99, IT Carcinogenicity Protocols and Dose Selections (1 volume)
027, 5-14-99 (1 volume)
028, 5-18-99 (1 volume)
Information to sponsor: Yes ( ) No (X)
Sponsor (or agent): Lilly ICOS LLC, c/o/ Lilly Research Laboratories
Manufacturer for drug substance: [redacted]

Drug:

Code Names: IC 351 (ICOS); LY 450190 (Lilly)
Chemical Name: (6R-trans)-6-(1,3-benzodioxol-5-yl) 2,3,6,7,,12,12a, hexahydro-2-methyl –pyrazinol[1,2:1,6] pyrido[3.4-b]indole-1,4-dione
CAS Registry Number: 171596-29-5
Molecular Formula: $C_{22}H_{19}N_3O_4$ Molecular Weight: 389.41
Structure:



Relevant INDs/NDAs/DMFs: NDA 20-895 Viagra;
IND [redacted]

Drug Class: Phosphodiesterase Type V Inhibitor

Indications: Erectile dysfunction (ED); [redacted]

Clinical formulation: 5, 10, and 20 mg tablets

Route of administration: Oral

Proposed clinical protocol or use: Sponsor has submitted draft protocols for two 12-week Phase III protocols ([redacted] -LVBM and [redacted] -LVBK) . [redacted] LVBM is the pivotal Phase III study to be conducted in the US with doses of 5 and 10 mg. An identical study will be conducted outside the US. The second study is a special population study to be conducted with 5, 10, and 20 mg doses in diabetic men with ED.

Previous clinical experience: Two Phase II studies have been completed. A European study was conducted with doses of 10, 25, 50 and 100 mg/day for 21 days in 294 men with ED. The major side effects were headache, back pain, myalgia and dyspepsia/nausea. A second 21-day Phase II study was conducted in the U.S. using doses of 5, 10, and 25 mg/day, prn .

Disclaimer – Numerous tables were taken directly from the submission to reduce retyping. Sponsors data is identified by specific reference.

TABLE OF CONTENTS

Introduction and drug history: IC 351 is a phosphodiesterase type V inhibitor, like Viagra, indicated for prn use in men with both organic and psychogenic erectile dysfunction. Like other phosphodiesterase inhibitors, the major toxicity is drug-induced vasculitis. IC 351 appears to be the most toxic of the PDE V inhibitors with respect to inducing vasculitis in dogs. The drug was originally developed by [redacted] stopped development after observing severe drug-induced vasculitis in dogs in the one and six month studies. The original submission by ICOS Corporation was placed on hold because daily dosing with up to [redacted] was originally proposed. The sponsor now expects efficacy with prn doses up to 10 mg. The lower dose levels produce parent drug AUC exposures in men which are 10-20 times lower than the AUC exposures in dogs which are associated with vasculitits.

Studies reviewed within this submission: The sponsor has provided the protocols and rationale for dose selection for the 2 year rat and 2-year mouse carcinogencicity studies which are ongoing. The 13-week mouse and 26-week rat studies used to support the dose selections were submitted in serial #022 (3-31-99) and are also reviewed herein.

Studies not reviewed within these submissions: N/A

ANCILLARY PHARMACOLOGY STUDIES; #1

Study Title: Potency and Selectivity of IC351 in an In Vitro Phosphodiesterase Activity Assay
Study No: 98-0001-11
Amendment #022, Vol # 11.1, and page #: 108
Conducting laboratory and location: ICOS Corporation, Bothell, WA
Dates of study : Jan 27-Feb 13, 1998
GLP compliance: No
QA- Report Yes () No (X)
Lot #: QEDI 18QSO497
Methods: IC 351 at concentrations from 0.3 nM to 10 uM was assessed in vitro for its inhibitory activity on human recombinant enzymes PDE 1A, 1B, 1C, PDE 2, PDE 4A, 4B, 4C, 4D, PDE 6, and PDE 7.
Results: The concentrations of IC351 producing 50% inhibition of enzymatic activity (IC_{50}) for the various PDE isozymes are presented in table 1 taken directly from the submission (vol 11.1, p 124). The IC_{50} of IC 351 for human recombinant PDE V was approximately 1 nM.

Table 3.1: Selectivity of IC351 for Human Phosphodiesterases (PDEs)

PDE	N[1]	IC_{50} ± SE (μM)[2]	Selectivity Ratio
1A	4	19 ± 4	18, 000
1B	4	20 ± 4	19,000
1C	4	10 ± 2	9,500
2	4	46 ± 8	44,000
3A	8	40 ± 9	38,000
3B	8	19 ± 5	18,000
4A	5	28 ± 5	27,000
4B	5	21 ± 4	20,000
4C	5	22 ± 4	21,000
4D	5	12 ± 3	11,000
5	8	0.00105 ± 0.00006	1
6	10	0.73 ± 0.12.	700
7	4	44 ± 7	42,000

[1] Number of independent data sets analyzed

[2] IC_{50} for combined data sets ± standard error of the fit

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